THE HEMICELLULOSES OF DOUGLAS FIR

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PART I

ABSTRACT

The Douglas fir holocellulose was prepared by Wise's(8) modification of Jayme's sodium chlorite procedure(9). The hemicelluloses were extracted using 24% potassium hydroxide followed by 17% sodium hydroxide containing 5% boric acid.

Meier(31) reported that barium hydroxide can be used to purify polysaccharide mixtures. The mode of action presumably being the formation of an insoluble complex with the cis-hydroxyl groups of the mannose or galactose integers. By using this method of purification Timell(30) isolated a galactoglucomannan from a 24% potassium hydroxide extract of eastern hemlock holocellulose. Following Timell's scheme the attempt to isolate a galactoglucomannan from Douglas fir did not proceed with the facility suggested. Two purifications by Meier's procedure failed to remove the xylose containing polysaccharide. Further batches of freshly prepared holocellulose were extracted and repeated attempts at the isolation and purification of a galactoglucomannan are at present being pursued.

The addition of barium hydroxide not only removed the galactoglucomannan fraction but also purified the xylan which remained in solution. Four barium hydroxide treatments followed by two purifications by means of Fehling's solution gave an arabino-4-O-methyl-D-glucuronoxylan freed from galactose. Previous attempts at the purification of this type of polysaccharide from softwoods have failed to remove the galactose residues.

A glucomannan fraction was extracted using 17.5% sodium hydroxide containing 5% boric acid. Four fractionations by the barium

hydroxide procedure failed to remove the xylose residues. Two further fractionations via the copper complex removed the last traces of xylose but the fraction still contained about 8% galactose. This is not unusual for it has been reported that the majority of gymnosperm glucomannans contain at least 4% galactose. The glucose to mannose ratio is approximately 1:3.5 which is in the order of a true glucomannan.

It appears that the possibility should be seriously considered that the galactose residues present in the so called "glucomannans" are actually integral parts of these polysaccharides. Additional experimental data in the near future will undoubtedly serve to solve the problem.

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TABLE OF CONTENTS

LIST OF FIGURES, TABLES ETC.

HISTORICAL INTRODUCTION

Polysaccharides other than cellulose have long been recognized in wood. Crude preparations described as wood gum were obtained by Tollens(1) by precipitations with alcohol from cold alkaline extracts of wood.

The term hemicellulose was first applied by Schylze(2) to the dilute alkali soluble polysaccharide of plant materials. He attempted to divide the carbohydrates of plant tissues according to their roles. The insoluble and difficultly hydrolyzable polysaccharide (cellulose) had a structural role. The easily hydrolyzable polysaccharide (starch) was a reserve which could later be mobilised. Between these two there fell a group of substances hydrolyzable by dilute acids and extractable by alkali to which he gave the name 'hemicellulose'. On hydrolysis of the hemicelluloses, sugars such as xylose, arabinose, glucose, mannose and galactose were obtained, whereas cellulose gave rise only to glucose. The hemicellulose group was considered by him to be chemically and structurally related to cellulose but to have possible reserve functions as well. Schulze and his co-workers concerned themselves primarily with the hemicelluloses of seeds and green tissues rather than those of wood. They considered the hemicelluloses to be simple hexosans and pentosans or frequently hexopentosans.

To clarify the terminology a little further, hemicelluloses which on hydrolysis with dilute acid give only glucose, galactose or xylose are referred to as glucans, galactans and xylans respectively. Hemicelluloses which, for example, give a mixture of glucose and mannose,

the major component (mannose) is the suffix — in this case glucomannan.

The modern period opened with the work of Schryver(3) who by alkaline extraction of American white oak, obtained products resembling the hemicelluloses of Schulze. On hydrolysis a mixture of xylose, arabinose, mannose and galactose was obtained, the pentose sugars predominating. To Schryver is due the recognition of the presence of uronic groups in hemicellulose preparations from wood. O'Dwyer(4), working with the same material, effected a separation of the material extracted by alkali, a portion precipitated by simple acidification being termed 'hemicellulose A' and a portion which could be obtained from the filtrate only by addition of alcohol being termed 'hemicellulose B'. This fractionation procedure has been followed in various modifications by almost all subsequent workers.

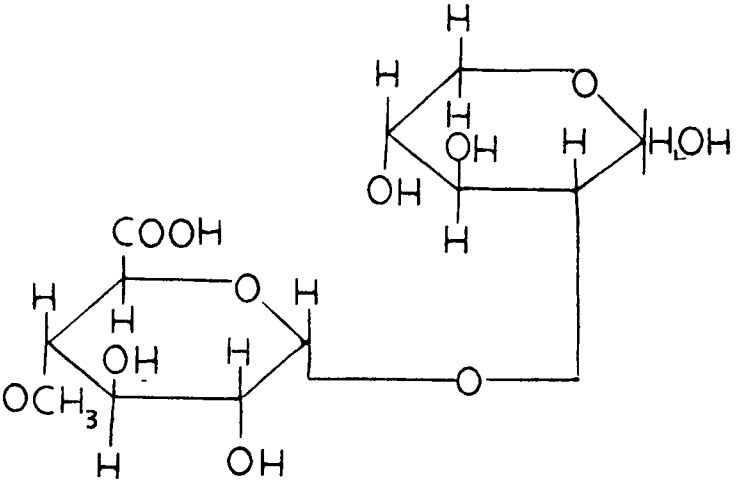
O'Dwyer(5) first identified glucuronic acid in the 'hemicellulose A' fraction from beechwood, and Anderson(6) demonstrated that the uronic content of wood is reduced by treatments that extract the hemicelluloses. These and parallel investigations on other sources have established the ubiqity of the hexauronic acids in crude hemicellulose preparations. In most cases the uronic content is not very high and in woods the hexauronic acid when identified has been found to be glucuronic acid or a monomethoxy derivative thereof. These are seldom isolated as the methoxyuronic acid but are more frequently found as methyl ethers of disaccharides or trisaccharides, the so called aldobiouronic and aldotriouronic acids. These disaccharides are formed by a uronic acid glycosidically linked with a simple sugar. The exact structure of the aldobiouronic acid has been studied in detail within the past few years. In most cases the structure has been found to consist of 4-O-methyl-D-glucuronic acid

linked α to D-xylose (generally through the 2 position, see formula I). Such aldobiouronic acids are surprisingly resistant to hydrolysis.

Hemicelluloses of varying degrees of solubility can be removed from an aqueous alkali extract of plant material by precipitation with alcohol. If the extraction is made on a lignocellulosic material, the precipitated hemicelluloses are contaminated with ligneous material, the removal of which involves a prolonged refining process. On the other hand, both the readily soluble and the more insoluble hemicelluloses are present in holocellulose.

Large samples of holocellulose may now be prepared either by the U.S. Forest Products Lab. Method(7) or by Wise, Murphy and D'Addieco's modification(8) of the Jayme's sodium chlorite procedure(9) without great loss of carbohydrate. However, when structural studies are to be made on uronic or aldobiouronic acid fractions of hemicelluloses of a hardwood, it may be best to extract the hemicelluloses directly from the sample with alkali without preconversion into holocellulose. This avoids the complication of aldonic acid formation.

By far the most commonly used holocellulose method is the chlorite method originally described by Jayme(9) but developed to its present day form by Wise and co-workers(8). This method gives holocelluloses in near-quantitative yields in a relatively short time but the results have been criticized by Timell(10, 11), who found that the chlorite method gives a more highly degraded product than does the chlorine-ethanolamine method introduced by Van Beckum and Ritter(12). The latter method has been developed as a TAPPI Standard(13). A promising but relatively new and less



I

Fig. 1. Typical structure of an aldobiouronic acid.

well known method is the peracetic acid method, first described by Poljak(14) and further developed by Haas, Schoch and Strole(15). Other methods using nitric acid(16, 17), monoethanolamine(18) and hypochlorite(19) have been suggested but have not won acceptance.

The preparation of holocellulose(20) fibres from Loblolly pinewood by the chlorite, chlorine-ethanolamine and peracetic acid methods were compared with respect to yield, degradation and D.P. In all cases, retention of carbohydrate was good, with some loss of xylan in the case of chlorine-ethanolamine and of mannan in the case of peracetic acid. However, the peracetic acid method modified by a subsequent sodium borohydride step gave a superior holocellulose.

In the course of any rigorous characterisation of these polysaccharides, there are three separate and essential stages, (a) isolation from suitable plant source, (b) fractionation into homogeneous components, (c) proof of structure of individual components. Fractionation by precipitation and extraction processes depends on solubility difference between the components. With hemicelluloses, solubility may be variably influenced by average molecular weight, distribution of molecular weight, shape, type and configuration of functional groups, homogenuity of composition and relationships between the cell wall components. Consequently, fractionation should not be governed by any single one of these solubility factors but instead by an average of the plus and minus features. It is apparently for this reason that overlapping or tailing of features is experienced and it is only by the most tedious and painstaking procedures that high purity is actually achieved and only by large sacrifices in yield and time.

Ordinarily, fractionation by extraction is not as efficient as by precipitation from a homogeneous solution. This is because dissolution is controlled more by accessibility, diffusion, membrane barriers, swelling restrictions etc. than by molecular properties.

In most of the reported studies on the isolation of hemicelluloses, dilute to concentrated aqueous solution of either sodium or potassium hydroxide has been employed as the extraction medium. In many instances, the use of potassium hydroxide was featured because the potassium acetate formed on neutralisation of the alkaline extract with acetic acid was more soluble in the alcohol that was used to precipitate the hemicellulose.

Experiments by Hamilton(21) on the extraction power of lithium, sodium and potassium hydroxide solutions for hemicelluloses showed that lithium and sodium hydroxides can remove all the mannose-containing polymers, whereas, potassium hydroxide is less effective. The size of the various alkali cations contribute to the ability to swell and dissolve components of the wood system. Another important factor which determines the swelling and dissolving action is the degree to which the ions and ion pairs are hydrated. Lithium and sodium are probably very similar in their ratios of ions to ion pairs and also in their type of hydration shells, whereas, potassium is different. It has an entirely different ratio of hydrated ions to hydrated ion pairs as well as a different degree and type of hydration shell.

The addition of borate salts to alkaline extractants has been used frequently to facilitate the removal of mannans(22), presumably by means of the complex formed between the borate ion and the cis-hydroxyl groups.

The removal of galactose containing polysaccharides can be achieved by either sodium or potassium hydroxide because according to many workers galactose containing polysaccharides are known to be located in the outside cell walls. In fact Wise(23) isolated a polysaccharide containing galactose, arabinose and xylose in the ratio of 90:9:1 from Douglas fir wood using boiling water as extractant.

During the last four years many glucomannans have been isolated from the wood of various gymnosperms. They have all been found to contain linear or slightly branched chains of (1 — 4) linked β-D-mannose and β-D-glucose residues usually in the ratio of 3 to 4:1 and probably distributed at random. Their number-average degrees of polymerisation fall within a range of 70 to 130.

The re-examination of glucomannans previously investigated by Timell(24) showed that those from softwoods all contained galactose residues. The delignification of a glucomannan from white pine followed by repeated precipitation with barium hydroxide failed to eliminate all galactose residues in this polymer. The following table summarises the characteristics of some of the glucomannans so far isolated from gymnosperm woods.

Species	Galactose %	Mann/Glu
Engelmann Spruce	3.5	3.3
White spruce	3.0	3.1
Jack Pine	2.9	2.9
Scots Pine	4.0	3.5

Species	Galactose %	Mann/Glu
Loblolly Pine	3.7	2.7
Eastern Hemlock	4.0	3.0
Amabilis Fir	3.0	3.3
Ginkgo Biloba	4.2	3.6

The average ratio of galactose:glucose:mannose is 1:8:24. In subsequent studies, the isolation of 2:3:4:6-tetra-O-methyl-D-galactose from a methylated glucomannan was reported. The galactose was assumed to occur as a terminal, nonreducing end group in the hemicellulose. A tetra O-methyl-galactose was also isolated by Dutton and Hunt(25) from Sitka spruce. These workers suggested that it was derived from a short chain polysaccharide easily removed on dialysis of the methylated hemicellulose.

It appears that the possibility should be seriously considered that galactose residues present in the so called 'glucomannans' are actually integral parts of these polysaccharides and should be classed as a galactoglucomannan. Additional experimental data in the near future will undoubtedly serve to solve the problem.

The occurrence of galactoglucomannans in softwoods has been referred to on several occasions. These polysaccharides usually have a ratio of glucose:galactose:mannose in the order of 1:1:3. Data obtained by the methylation and periodate oxidation techniques suggested the presence of a framework of β-(1 — 4) linked D-glucose and D-mannose residues, to which single, terminal side chains of D-galactose were directly attached by (1 — 6)-glycosidic bonds.

The isolation of a β-(1 — 4) linked galactan by Bouveng and

Meier(26) has cast suspicion on the homogenuity of the triheteropolymer. Meier(27) has recently reported the isolation of a 6-O-β-D-galactopyranosyl-D-mannose and an O-β-D-galactopyranosyl-(1 — 6)-O-β-mannopyranosyl-(1 — 4)-D-mannose. The isolation of these constitutes the first conclusive evidence that galactose and mannose are chemically combined in wood. These oligosaccharides could well have originated from a galactoglucomannan but Meier prefers to consider them as derived from a mixture of equal parts of a glucomannan and a galactomannan.

Analysis of the hemicelluloses of wood indicates the presence of a high proportion of D-xylose unites in association with those of a methyl ester of a hexuronic acid. These xylans are usually composed of chains of (1 — 4) linked β-D-xylopyranose residues carrying (through position 2) a single 4-O-methyl-D-glucuronic acid residue attached as a side chain. Most wood xylans have that general type of linkage and differing slightly in average molecular size and in proportion of hexuronic acid residues attached as side chains. In general, the proportion of residues of 4-O-methyl-D-glucuronic acid is higher in the softwood xylans (15-20%) than those of hardwoods (8-15%).

In addition to end groups of 4-O-methyl glucuronic acid, some xylose containing polysaccharides contain quantities of L-arabinose furanoside end groups. These are generally found in softwoods and are referred to as arabinoglucuronoxylans. It would be in accordance with previous experimental results to refer to the xylose containing polysaccharide from Douglas fir as an arabinoglucuronoxylan. However, as no structural determinations have been carried out, we shall refer, for convenience, to

this fraction as a xylan.

For the identification and separation of the acid hydrolysates, earlier workers(28) had to rely an either the preferential crystallisation of the different sugars from the mixture or characterisation of a sugar in the mixture by a definite, well known derivative. Today a quick preliminary method is available in paper chromatography which can also serve as a quantitative method of analysis. Elution of the various zones with water and treatment of the solution with phenol and sulphuric acid(29) gives a reproducable colorimetric method of analysis. Other quantitative methods utilised the spot obtained by spraying the chromatogram with a detecting agent.

Acid hydrolysis of the hemicellulose followed by separation and identification of the individual sugars, shows the sugars present and their proportion in the mixture. It does not, however, tell the order of attachment of the sugars to one another or the position of the links between the sugars.

OUTLINE

During the preliminary experiments on this wood, batches of chlorite holocellulose were prepared using a copper immersion heater. The holocellulose appeared green in color, characteristic of copper salts, rather than the expected bleached white due to the action of chlorine.

The extraction of hemicelluloses from the holocellulose proceeded with much difficulty. The fraction extracted with 17% sodium hydroxide containing 4% boric acid amounted to only 1.2% of the total wood. The sugar ratios of this fraction after two fractionations with barium hydroxide showed the unusual ratio of glucose to mannose of 3:1. The expected ratio being the inverse, 1:3. Due to fractionation difficulties and low yields of fractions, a new batch of holocellulose was prepared using live steam as the heating source.

During the past few years, much of the work carried out in this laboratory has been associated with the isolation and structural determination of hemicelluloses(34, 35). In 1958 Meier(31) discovered that barium hydroxide could be used as a fractionating agent and in 1961 Timell(30) achieved considerable success in applying this procedure to the isolation and purification of hemicelluloses from certain gymnosperms. In this thesis, the procedure followed by Timell is applied to the isolation and purification of hemicelluloses from Douglas fir.

The initial scheme of extraction and fractionation of the hemicelluloses from Douglas fir followed in this work is outlined by Timell(30).

The Douglas fir holocellulose was prepared by the method of Wise et. al.(8). Timell, however, points out that losses of galactose containing polysaccharides could be anticipated using this method of delignification.

The holocellulose extracted with a 24% aqueous potassium hydroxide solution yielded 162g of a mixture of hemicelluloses designated D.F.X_1 (Douglas fir $xylan_1$). The alkaline solution was treated with sodium borohydride to reduce any free or potential aldehyde functions so as to confir immunity to alkaline oxidation. Acidification of a small sample gave no 'hemicellulose A' as defined by O'Dwyer(4). According to the outline of Timell(30), one of the hemicelluloses extracted by potassium hydroxide is a galactoglucomannan which could be removed by treatment with barium hydroxide according to Meier(31). Subsequent treatment with barium hydroxide yielded a soluble (D.F.X_2) and an insoluble fraction. D.F.X_2 was further fractionated by this method to give D.F.X_3 which in turn was fractionated to give D.F.X_4. At this stage, the amount of barium hydroxide complex was small suggesting that most of the galactoglucomannan had been removed. The three barium hydroxide precipitates thus obtained were combined and designated D.F.GGM_1 (Douglas fir $galactoglucomannan_1$). Extreme difficulty has been experienced in purification of this component and attempts to free the galactoglucomannan from xylose is still being pursued.

Paper chromatographic examination of the acidic hydrolyzates of D.F.X_2, D.F.X_3 and D.F.X_4 showed the presence of galactose, glucose, mannose, arabinose and xylose. No quantitative estimations were carried out on hemicellulose D.F.X_2 and D.F.X_3 because from the relative size and intensity

of the spots, the proportion appeared to be changing gradually. D.F.X_4 showed the presence of considerable amounts of hexoses. The ratio of glucose and galactose:arabinose:xylose was 2.5:3.5:19.5. A further treatment with barium hydroxide gave D.F.X_5 which had a ratio of glucose and galactose:arabinose:xylose of 1:4:21.5. It is noteworthy that in both fractions the ratio of arabinose to xylose remains approximately constant.

Two additional fractionations via the copper complex by treatment with freshly prepared Fehling's solution(32) gave hemicelluloses D.F.X_6 and D.F.X_7. Fraction D.F.X_6 was not examined chromatographically. Sugar estimations on D.F.X_7 indicated the removal of the last traces of hexoses. The ratio of arabinose to xylose was found to be approximately 1:5.5.

The residue remaining after the extraction with potassium hydroxide was thoroughly extracted with 17.5% sodium hydroxide containing 4% boric acid. The material extracted at this stage was called 'crude glucomannan' designated D.F.GM_1. The alkaline extract was treated with sodium borohydride to prevent alkaline degadation as mentioned earlier. D.F.GM_1 was fractionated three times by the barium hydroxide procedure to give D.F.GM_2, D.F.GM_3 and D.F.GM_4. In all cases, with the exception of D.F.GM_1, sugar estimations were carried out. In order to bring about a satisfactory separation between galactose and glucose by paper chromatographic procedures, the development time was such that the fraction corresponding to xylose was allowed to run off the paper and consequently no xylose ratio is reported but where present , is indicated.

The fraction, D.F.GM_4 still showed the presence of xylose and galactose and consequently purifications via the copper complex were carried

in the hope that the last traces of these sugars would be removed. At the D.F.GM_5 stage, the fraction was split into two portions, (a) insoluble in hydrochloric acid, (b) soluble in hydrochloric acid. For full details, see experimental page 23 . At that time it was felt that some sort of separation may have been effected, but as the work is not yet complete, the possibility is still being pursued. For sugar determinations to date on the various stages of purification, see table II. It is noteworthy that in fraction D.F.GM_6 the last trace of xylose has been removed and that the mannose:glucose ratio for all stages of purification is of the order of a true glucomannan.

The presence of galactose in these fractions is not unusual since all other softwood glucomannans have invariably been found to contain galactose units, usually from 3% to 4%. See table on page 7 . However, a glucomannan from black spruce has recently been isolated free of galactose by Pierre(33).

At the present time further batches of Douglas fir holocellulose are being extracted in an attempt to isolate a pure galactoglucomannan and to repeat purifications on the glucomannan fraction in the hope that the galactose residues may be removed.

EXPERIMENTAL

Unless otherwise stated, all the sample weights are quoted on an oven dry basis. Samples being dried at 100°C for 4 hours.

I. Paper Chromatography.

The solvent system which gave the best separation of sugars was found to be ethyl acetate-pyridine-water (8:2:2 upper phase). Separations were carried out on Whatman No.1 filter papers by the descending technique. The spray reagent was p-anisidine-trichloroacetic acid. For quantitative analysis samples were prepared as follows:- approximately 20mg samples were hydrolysed with 1N sulphuric acid (15ml) on a steam bath for 12 hours. The samples were then treated with Duolite A-4 resin to remove all anions and then with Amberlite IR-120 to remove all cations. The deionised solution was then concentrated to approximately 0.5ml and chromatographed as suggested above. The development time varied with the ambient temperature and with the solvent batch. The zones corresponding to the various sugars were eluted with water (25ml). Aliquots (2ml) were taken from this solution and phenol (100 l) and concentrated sulphuric acid (5ml) added. The intensity of the color produced, as measured on a Bauch and Lomb colorimeter, is proportional to the concentration of sugar. The analyses were done in triplicate. Standard curves used for these estimations are shown on page 24, 25 and 26.

II. Preparation of Holocellulose.

The wood from Douglas fir (1000g, total lignin, 32 to 34% —

determined by TAPPI Standard G.8) was suspended in water (141) at 75°-80°C. Glacial acetic acid (100ml) and sodium chlorite (300g) were added and the reaction allowed to proceed for 1 hour. The same amounts of reagents were added every hour for an additional period of three hours. The partly delignified product was allowed to settle and was washed several times with tap water by decantation. The solids were transferred to the centrifuge basket and washed free from acid. Yield: 835g corresponding to 83.5% of the wood, residual lignin 10%.

III. Extraction of a Xylan Fraction.

Holocellulose (835g) prepared by the chlorite method was shaken with 24% aqueous potassium hydroxide (81) solution for 18 hours. The solution was filtered and the solid material washed with cold water (31). To the alkaline extract and water washings was added sodium borohydride (2g) and the mixture allowed to stand overnight.

IV. Purification of Xylan.

To the alkaline extracts and water washings from III was added a 5% barium hydroxide solution (111) dropwise over a period of 2 hours. The precipitate did not form until approximately three-quarters of the barium hydroxide had been added. The precipitate I was collected on the centrifuge, washed with dilute alkali and set aside. The filtrate and washings were concentrated to 4 litres and poured into alcohol (151) containing glacial acetic acid (31). The precipitate D.F.X_2 was collected on the centrifuge, washed with alcohol and redissolved in 10% sodium hydroxide solution (51). The small amount of material which was insoluble in alkali was discarded. To this alkaline solution was added a 5% barium

hydroxide solution (6l) over a period of 2 hours. The precipitate II was collected, washed and set aside. The filtrate and washings were concentrated and poured into four volumes of ethanol containing sufficient acetic acid to neutralise the base present. The precipitate D.F.X_3 was collected and again fractionated by the same method described above. The precipitate III was set aside. Sugar ratios were determined on D.F.X_4. The presence of hexoses warranted a further fractionation. The purification by the barium hydroxide method resulted in a small amount of precipitate suggesting that an alternative method of fractionation should be attempted. Sugar ratios determined on D.F.X_5 still showed the presence of hexoses.

The fraction, D.F.X_5 (60g) was dissolved in a 5% sodium hydroxide solution (2l) and freshly prepared Fehling's solution (300ml) was added. No precipitate resulted but on the addition of approximately 50ml of acetone the characteristic blue gelatinous copper complex was formed. The precipitate was collected, washed twice with alkali and the comples decomposed with a cold 1:1 mixture of acetic acid and hydrochloric acid and the mixture poured into three volumes of alcohol. The precipitate D.F.X_6 was collected in the usual manner and given a further fractionation by this method. D.F.X_7 was collected and dried by solvent exchange (ethanol—ethyl ether—pet. ether). Yield: 15g corresponding to 1.5% of total wood. Sugar estimations showed the removal of the last traces of hexoses. Table I (p. 18) summarises the sugar ratios throughout each stage of purification. A flow sheet of the purification scheme is shown on page 19.

V. Isolation and Purification of a Galactoglucomannan.

The barium hydroxide complexes (precipitate I, II and III) collected

Table I. Summary of sugar ratios at the different stages of purification of the xylan fraction.

Hemicellulose Fraction	Gal.	Glu.	Man.	Arab.	Xyl.	Xyl./Arab.	Method of Purification
D.F.X_4	1.0	1.6	nil	3.4	19.4	5.7	Barium Hydroxide
	1.3	1.0		4.4	22.0	5.0	
D.F.X_5	3.0	1.0	nil	16.0	86.0	5.4	Barium Hydroxide
D.F.X_7	nil	nil	nil	1.0	6.4	6.4	Fehling's Solution
				1.0	7.2	7.2	

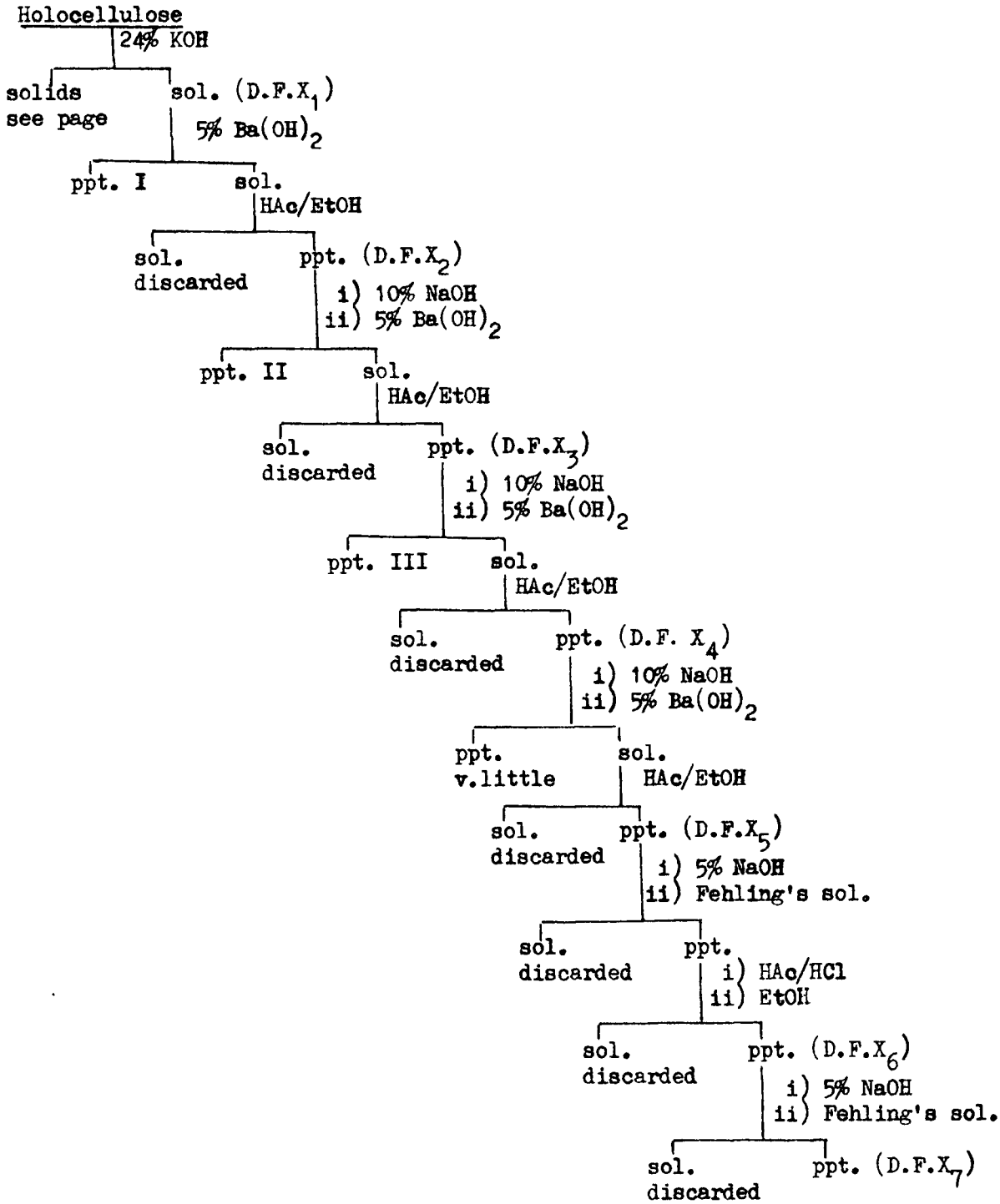


Chart 1. Flow sheet representing the purification of a xylan.

from the purification of the xylan under IV were decomposed using a cold 1:1 mixture of acetic acid and hydrochloric acid and the clear solution poured into three volumes of alcohol. The precipitated D.F.GGM$_1$ was collected on the centrifuge, washed once with alcohol and dissolved in 10% sodium hydroxide solution (21). Barium hydroxide solution (21, 5%) was added dropwise for a period of 2 hours. The precipitated complex was collected on the centrifuge and washed three times with water. The fraction, D.F.GGM$_2$ was collected in the usual manner. Paper chromatography of the hydrolyzate of a small sample of D.F.GGM$_2$ showed the presence of galactose, glucose, mannose, arabinose and xylose. The purification of this fraction is still in progress.

VI. Isolation and Purification of a Glucomannan.

The solid material remaining after the extraction with 24% potassium hydroxide was thoroughly washed and then treated with 17.5% sodium hydroxide containing 5% boric acid (61) for a period of 18 hours. The mixture was filtered on the centrifuge and the solid material washed with cold water (31). To the alkaline extracts and washings was added sodium borohydride (2g) and the mixture allowed to stand overnight. The hemicellulose D.F.GM$_1$ was collected by pouring this mixture into three volumes of alcohol containing sufficient glacial acetic acid to neutralise the base. Yield: 90g, corresponding to 9% of the total wood. The fraction, D.F.GM$_1$ was dissolved in a 10% sodium hydroxide solution (41) and a 5% barium hydroxide solution (41) added dropwise over a period of 1 hour. Unlike the galactoglucomannan, the precipitate formed immediately. After centrifuging and washing three times with dilute alkali, the complex was

Table II. Summary of sugar ratios at different stages of purification of the glucomannan fraction.

Hemicellulose Fraction	Gal.	Glu.	Man.	Arab.	Xyl.	Man./Glu.	Method of Fractionation
D.F.GM_2	1.0	2.8	9.2	nd	p	3.3	Barium Hydroxide
D.F.GM_3	1.0	3.3	9.5	nd	p	2.9	Barium Hydroxide
	1.0	3.6	10.7	nd	p	2.9	
D.F.GM_4	1.0	3.0	8.6	nd	p	2.9	Barium Hydroxide
D.F.GM_5	1.0	3.3	9.8	nd	0.6	3.0	Fehling's Solution
D.F.GM_5 sol.	1.0	3.8	20.0	nd	0.6	5.3	Fehling's Solution
D.F.GM_5 insol.	1.0	2.7	12.2	nd	2.0	4.5	Fehling's Solution
D.F.GM_6 sol.	1.0	2.6	14.3	nd	d	5.5	Fehling's Solution
	1.0	1.9	9.0	nd	a	4.7	
D.F.GM_6 insol.	1.0	14.0	33.0	nd	vf	2.4	Fehling's Solution
D.F.GM_7 sol.	1.0	2.8	12.4	nd	a	4.4	Fehling's Solution

D.F.GM_7 insol. is being examined at present.

nd = not detected; p = present; d = doubtful; a = absent; vf = very faint.

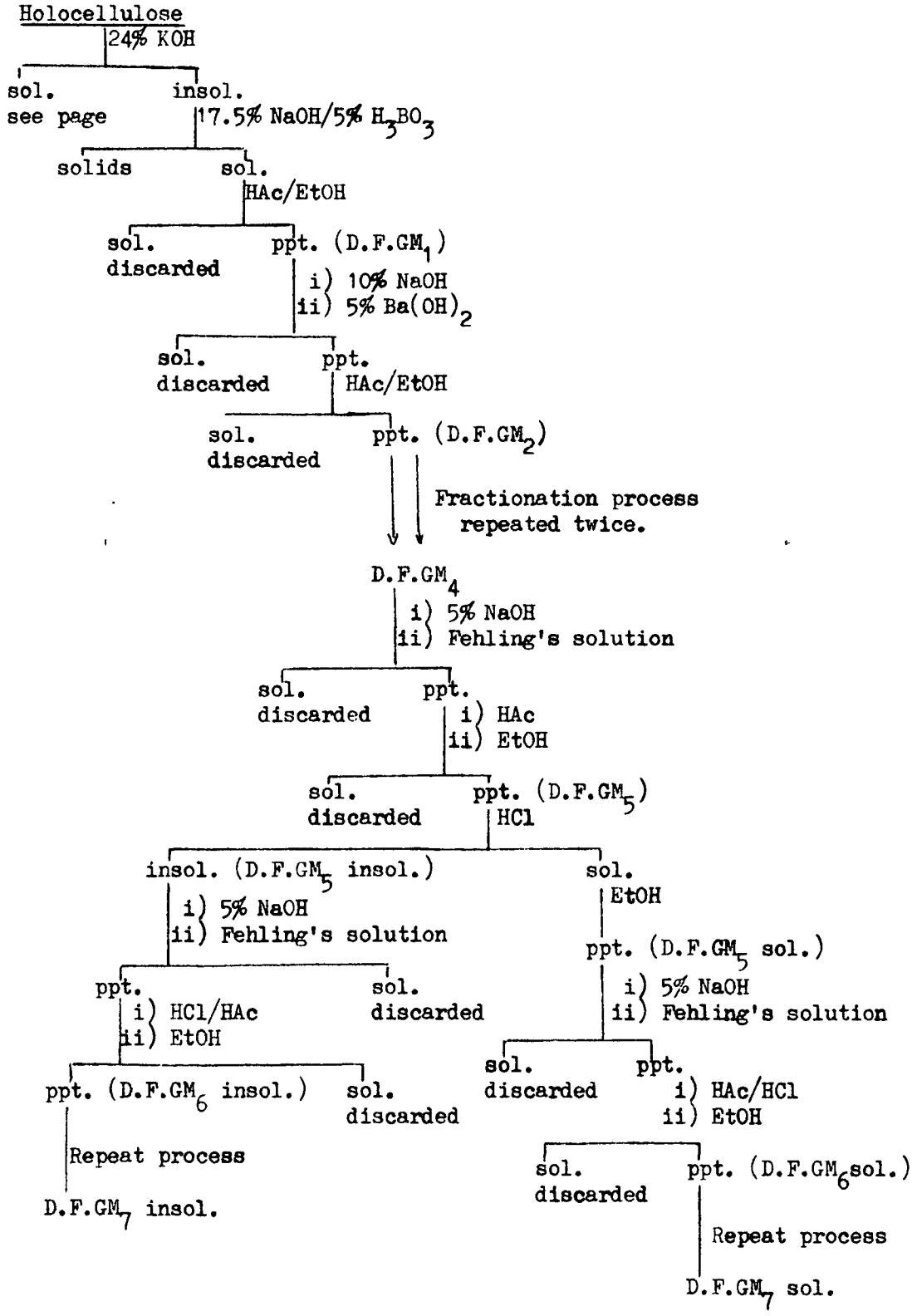
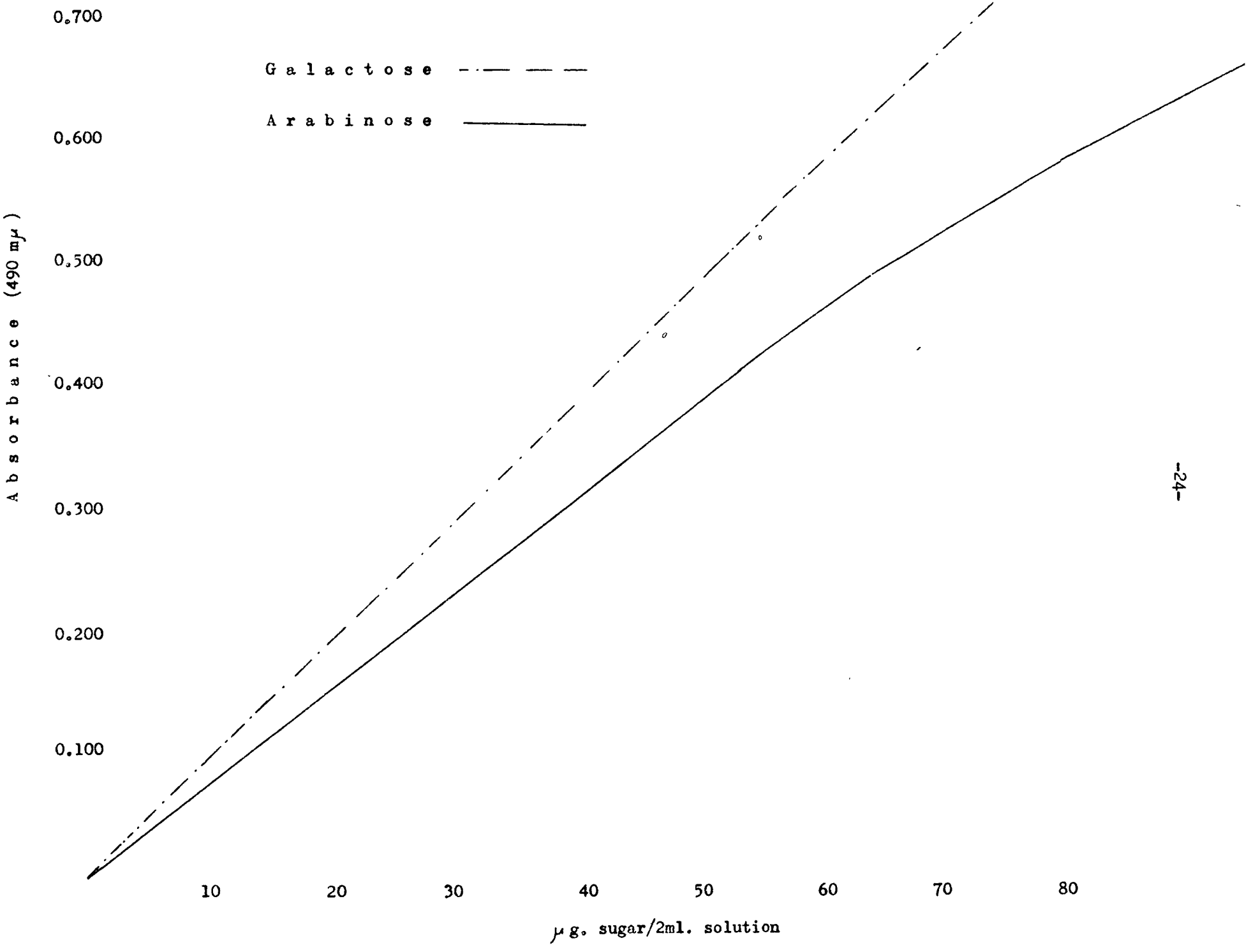
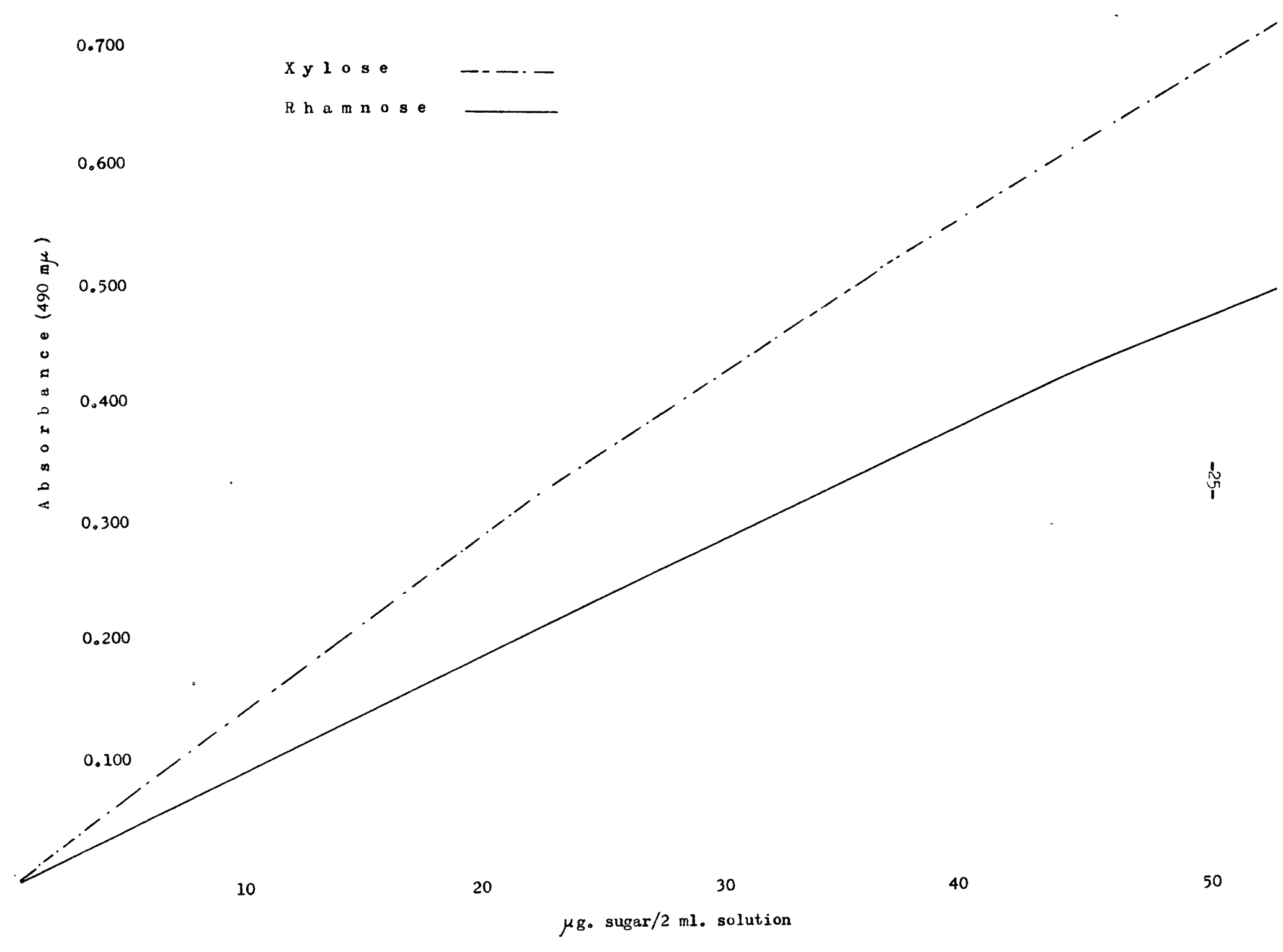


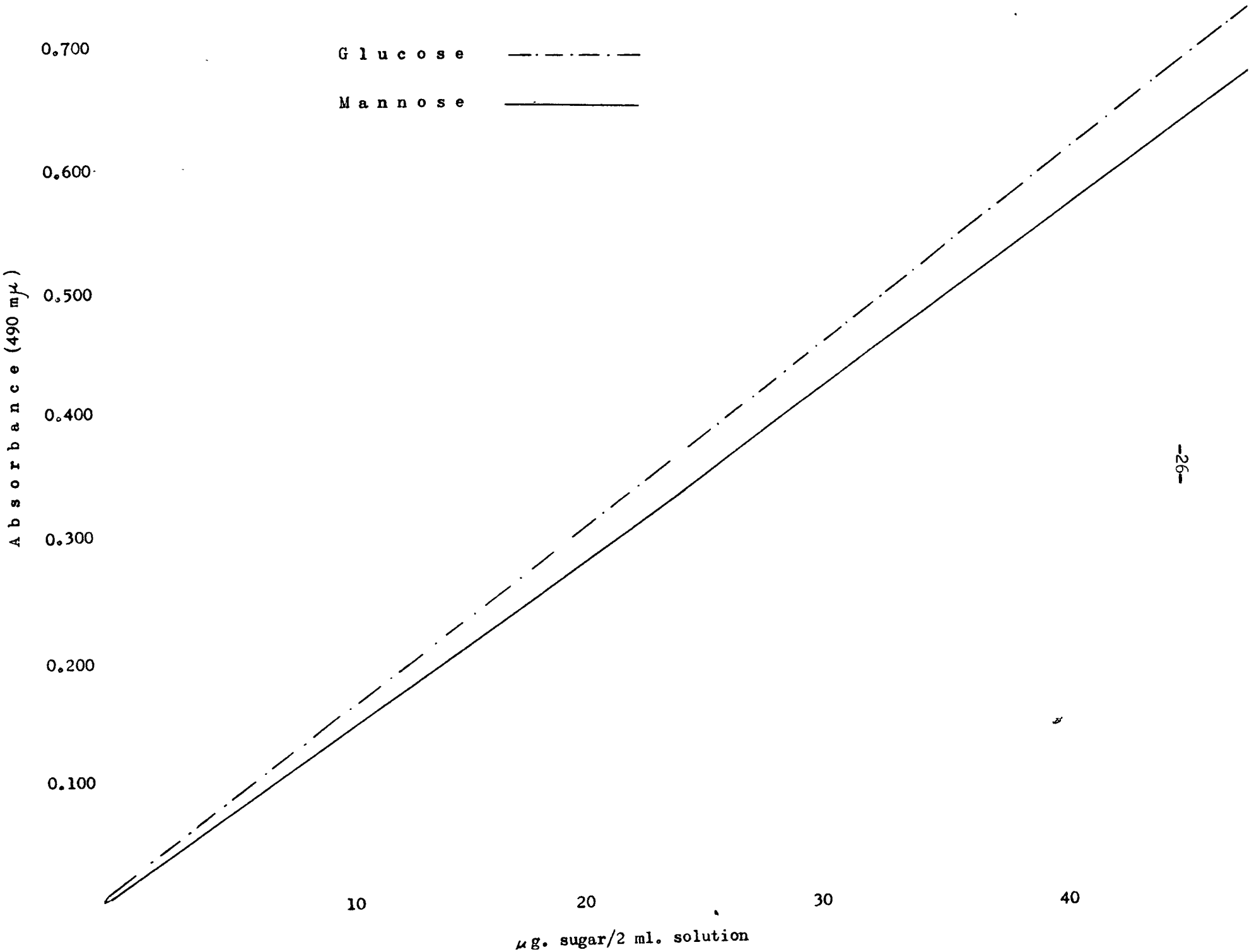
Chart 2. Flow sheet representing the purification of a glucomannan.

decomposed using ice and glacial acetic acid. The clear solution was poured into three volumes of alcohol. The precipitate D.F.GM_2 was collected as described above and fractionated a further two times. Sugar determinations were carried out on fractions D.F.GM_2, D.F.GM_3 and D.F.GM_4. See table II.

The fraction, D.F.GM_4 still contained small amounts of pentose. In an effort to remove the last traces of this sugar, fractionations via the copper complex were carried out. The sample, D.F.GM_4 was dissolved in a 5% sodium hydroxide solution (21) and freshly prepared Fehling's solution (200ml) added dropwise over a period of 1 hour. The precipitate was collected in the usual manner and decomposed using glacial acetic acid. On pouring the solution into three volumes of alcohol, a bluish precipitate D.F.GM_5 resulted suggesting that the copper complex had not been completely decomposed. The precipitate was collected by centrifuging and on treatment with hydrochloric acid gave a soluble and insoluble fraction. The white insoluble fraction was collected on the centrifuge and designated D.F.GM_5 insol. The clear supernatant was poured into three volumes of alcohol and the resulting precipitate designated D.F.GM_5 sol. Both these fractions were further purified via the copper complex to give D.F.GM_6 sol. and D.F.GM_7 sol., and D.F.GM_6 insol. and D.F.GM_7 insol. For summary of sugar estimations see table II. A flow sheet on page 22 summarises the purification scheme.


0.700
0.600
0.500
0.400
0.300
0.200
0.100
Galactose
Arabinose
Absorbance (490 mμ)
10
20
30
40
50
60
70
80
μg. sugar/2ml. solution


0.700
0.600
0.500
0.400
0.300
0.200
0.100
Absorbance (490 mμ)
Xylose
Rhamnose
10
20
30
40
50
μg. sugar/2 ml. solution


Glucose
Mannose
0.700
0.600
0.500
0.400
0.300
0.200
0.100
Absorbance (490 mμ)
10
20
30
40
μg. sugar/2 ml. solution

BIBLIOGRAPHY

(1) Wheeler, H.I., and Tollens, B., Ann., 254: 316 (1889).

(2) Schulze, E., Ber., 24: 2277 (1891).

(3) Clayson, D.H.F., Norris, F.W., and Schryver, S.B., Biochem. J., 15: 643 (1921).

(4) O'Dwyer, M.H., Biochem. J., 17: 501 (1923).

(5) O'Dwyer, M.H., Biochem. J., 20: 656 (1926).

(6) Anderson, E., J. Biol. Chem., 91: 559 (1931).

(7) Van Beckum, W.G., and Ritter, G.J., Paper Trade J., 104, No. 19: 49 (1937).

(8) Wise, L.E., Murphy, M. and D'Addieco, A.A., Tech. Assoc. Papers, 29: 210 (1946).

(9) Jayme, G., Cellulosechem., 20: 43 (1942).

(10) Timell, T.E., Svensk Papperstidn., 59: 1 (1956).

(11) Timell, T.E., Pulp and Paper Mag. Can., 60: 26 (1959).

(12) Van Beckum, W.G. and Ritter, G.J., Paper Trade J., 105, No. 18: 127 (1937).

(13) TAPPI Standard T9 m-54.

(14) Poljak, A., Angew. Chemi., 60: 45 (1948).

(15) Hass, H., Schock, W., and Strole, V., Das Papier, 9: 469 (1955).

(16) Kurschner, K., and Hoffer, A., Tech. Chem. Papier-U. Zell-Stoff-Fabr, 26: 125 (1929).

(17) Jayme, G. and Schorning, P., Papier-Fabr., 36, No. 24/25: 235 (1938).

(18) Wise, L.E., Peterson, F.C., and Harlow, W.M., Ind. Eng. Chem., Anal. Ed., 11: 18 (1939).

(19) Norman, A.G., and Jenkins, S.H., Biochem. J., 27: 818 (1933).

(20) Leopold, B., TAPPI, 44: 230 (1961).

(21) Hamilton, J.K., TAPPI, 40: 781 (1957).

(22) Jones, J.K.N., Wise, L.E., and Jappe, J.P., TAPPI, 39: 139 (1956).

(23) Wise, L., et.al., TAPPI, 36: 319 (1953).

(24) Timell, T.E., TAPPI, 44: 88 (1961).

(25) Dutton, G.G.S., and Hunt, K., J. Am. Chem. Soc., 80: 5697 (1958).

(26) Bouveng, H.D., and Meier, H., Acta Chem. Scand., 13: 1884 (1959).

(27) Meier, H., Acta Chem. Scand., 14: 749 (1960).

(28) O'Dwyer, M.H., Biochem. J., 16: 503 (1923).

(29) Dubois, M., Giles, K., Hamilton, J.K., Rebus, P.A., and Smith, F. Nature, 168: 167 (1951).

(30) Timell, T.E., TAPPI, 44: 88 (1961).

(31) Meier, H., Acta Chem. Scand., 12: 144 (1958).

(32) Salkowski, E., Z. Physiol. Chem., 34: 443 (1952).

(33) Pierre, K.J., M.Sc. Thesis, University of British Columbia, June (1962).

(34) Murata, T.G., M.Sc. Thesis, University of British Columbia, October (1960).

(35) Hunt, K., M.Sc. Thesis, University of British Columbia, April (1957).

PART II

ABSTRACT

In 1927, Levene and Meyer(9) claimed to have been able to prepare pentamethyl aldehydo-D-glucose by subjecting D-glucose diethyl mercaptal to two Haworth methylations followed by one Freudenberg methylation. The work described in this thesis is an attempt to prepare tetramethyl aldehydo-L-arabinose and tetramethyl aldehydo-D-xylose by methylating the diethyl mercaptal derivatives. From the methylation experiments carried out, it appears that the resistance of certain hydroxyl groups towards Purdie's reagents is much greater than anticipated.

A mixture of strontium oxide and strontium hydroxide was found to be as effective as silver oxide in methylation experiments.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

The methylation technique is of outstanding importance in the structural polysaccharide chemistry. The procedure involves the preparation of the exhaustively methylated polysaccharide, hydrolysis to a mixture of monomers and the separation and identification of these monomers. The original points of substitution will correspond to the unsubstituted hydroxyl groups in the monomeric methyl ethers. Identification of the monomers is aided by the comparison of their physical and chemical properties with standard material. An enormous amount of ingenious and time-consuming work has been devoted to the synthesis of the different methyl ethers of the naturally occuring sugars which are required for identification purposes. Many modifications of older methods and the development of newer methods of synthesis have been applied to the preparation of mono- and polymethylated sugars. Derivatives, such as sugar mercaptals, have been recognised for the past 68 years and are valuable intermediates in synthetic chemistry.

In 1894, the first sugar mercaptal or dithioacetal was prepared by Fischer(1). These compounds are formed by the reaction of mercaptans with aldehydes or ketones in the presence of acid catalysts (e.g. I). Unlike the acid catalysed reaction of alcohols with sugars which lead to the formation of cyclic glycosides, (e.g. II) these thiols yield dithioacetals.

These mercaptals have interesting properties in that they are necessarily acyclic since they no longer possess a carbonyl function. The formation of such acyclic structures frees an additional hydroxyl group

which is involved in the ring of the original sugar, thus simplifying many synthetic steps which may be involved in a reaction sequence.

In 1934, it was reported by Lieser and Leckzyck(2) that D-glucose diethyl mercaptal could selectively be methylated by the use of methyl iodide at 0°C. The formation of 2-O-methyl-D-glucose which is formed by hydrolysis affords an easy route to 2-O-methyl-D-glucose which is otherwise prepared more tediously. Surprisingly, this method fails when applied to diethyl mercaptals of D-galactose, L-arabinose and D-xylose.

Dutton and Yates(3) have reinvestigated these results and confirmed that only D-glucose diethyl mercaptal was reactive towards Purdie's reagents and attributed this observation to the insolubility of the other mercaptals in methyl iodide. They also concluded that when methylations were carried out in an inert solvent such as tetrahydrofuran, in which the sugar mercaptals are soluble, no peculiar selectivity of the reaction of D-glucose diethyl mercaptal was observed.

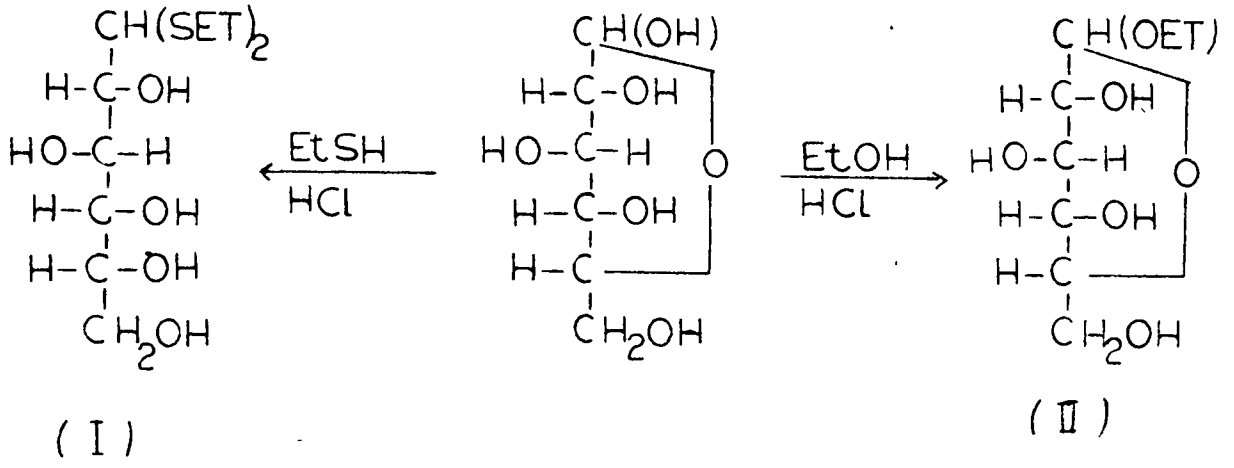
The synthesis of partially methylated monosaccharides requires preliminary protection of the hydroxyl groups which are to be left free in the final product, methylation of the unprotected hydroxyl groups and subsequent removal of the blocking groups, thus demanding, in many cases, several steps to synthesise partially methylated sugars. If, as has been demonstrated by Lieser and Leckzyck(2) and also by Wolfrom, Olin and Weisbalt(4) in the methylation of D-glucose mercaptals, selective methylations of any hydroxyl in sugar mercaptals are observed partial methylation of sugar mercaptals can supply a convenient route to the synthesis of partially

methylated sugars.

It is accepted that the most stable arrangement in a polyhydric alcohol is that in which the carbon chain assumes a planar zig-zag form(5) and D-glucitol and D-mannitol could be represented by structures(III) and (IV). From a comparison of the Fischer projection formula for these hexatols, it can be seen that the adjacent cis-hydroxyl groups in the Fischer projection formula (e.g. 4- and 5-hydroxyl groups of D-glucitol, and 2- and 3-hydroxyl groups of D-mannitol) are trans to each other in the zig-zag form.

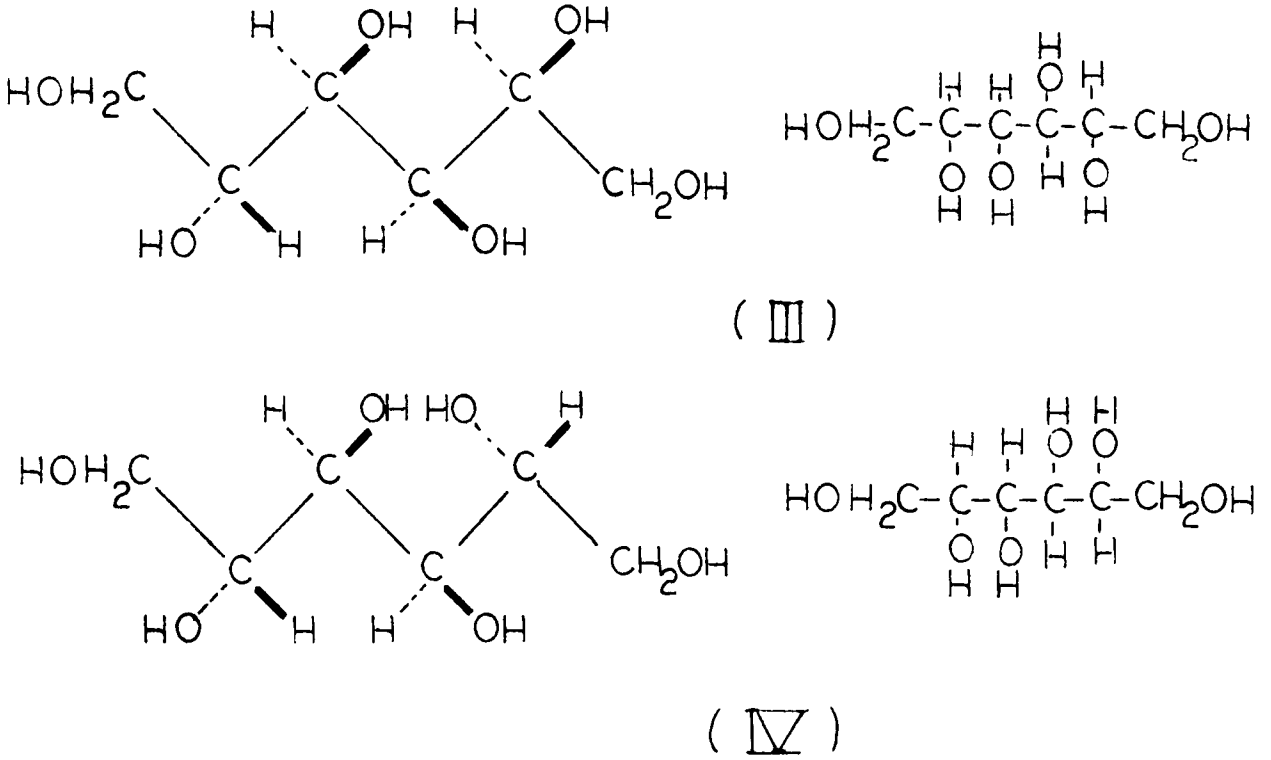
The conformation of sugar mercaptals are not known, but it would not seem unreasonable to suggest that an analogous zig-zag carbon conformation exists. The experimental results of many workers are explainable on the assumption that the polyhydric alcohols assume this zig-zag conformation and, similarly, many of the reactions of sugar mercaptals can be explained. Extensive work was carried out by Tanaka(6) on the relative reactivities of the hydroxyl groups of the mercaptals of D-glucose, D-mannose, D-galactose, L-arabinose and D-xylose towards methylation. Using the idea of the zig-zag conformation and the existence of intramolecular hydrogen bonding in sugar molecules(7) which has been recognised for some time, Tanaka(6) was able to explain the reactivities of some of the hydroxyl groups.

These results have shown that the 2-hydroxyl group has the highest reactivity towards methylation. This is explainable on the inductive effect of the two sulphur atoms attached to carbon-1. The acidic character of the 2-hydroxyl group has been demonstrated by Papadakis(8) who reported the



(I) (II)

Fig. 1. Formation of glycosides and mercaptals.



(III) (IV)

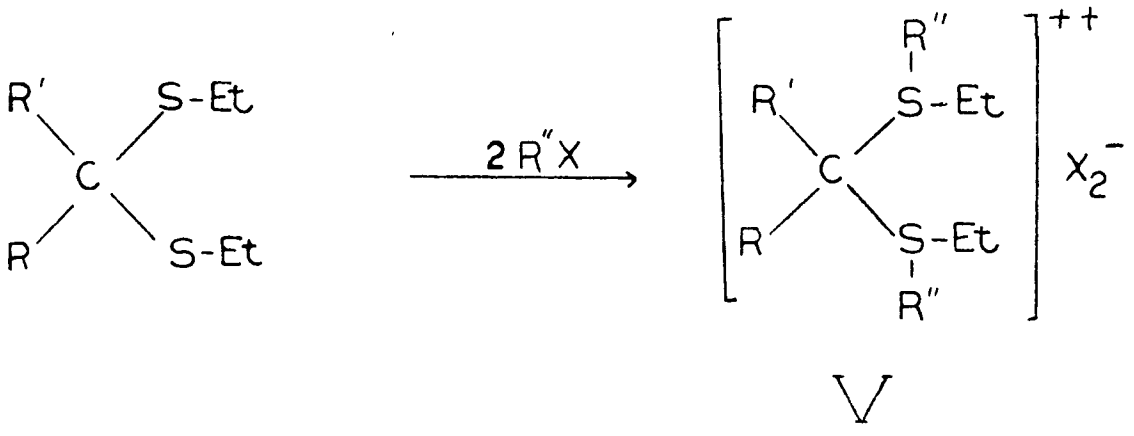
Fig. 2. Zig-zag structure of some glycitols.

synthesis of 2-O-methyl-D-glucose diethyl mercaptal by the action of methyl iodide on mono-O-sodio-D-glucose diethyl mercaptal prepared by Fischer(1) from the mercaptal and sodium ethoxide. The 3-hydroxyl appeared to have the next highest reactivity which could be explained by the inductive effect of the sulphur atoms.

It is also well known that alkyl sulphides can form stable sulphonium compounds with alkyl halides. Mercaptals of ordinary aldehydes also form bis-sulphonium derivatives with alkyl halides (e.g. V). Such a reaction probably occurs with sugar mercaptals, since some compounds having R_f values lower than the unsubstituted sugar mercaptal were observed in the preliminary work(3). These could possibly be the sulphonium compounds because polar compounds ususally travel slowly in paper chromatography. These sulphonium salts would enhance the acidity of the 2-hydroxyl group and the predominant methylation at the 2-hydroxyl group is equally well explained in terms of the inductive effect of this group. The reactivity of the other hydroxyl groups is explainable in terms of intramolecular hydrogen bonding.

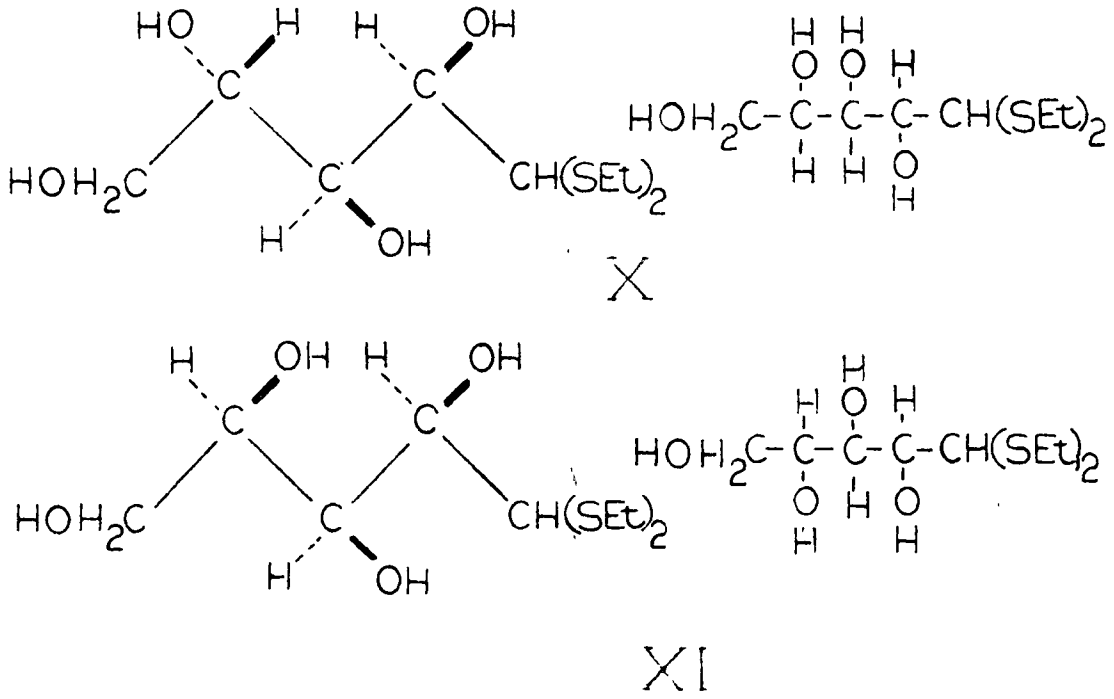
The stability of these mercaptals in basic solution makes them useful intermediates for keeping sugars in the acyclic form during methylation. It thus appears that these would serve as useful intermediates in the preparation of fully methylated sugars. The preparation of 2,3,4,5,6,-pentamethyl-D-glucose has been reported by Levene and Meyer(9) by hydrolysis of 2,3,4,5,6-pentamethyl-D-glucose diethyl mercaptal.

In investigating the possibility of preparing a fully methylated derivative of gluconic acid, Pryde(11) found that the reaction may be arrested



V

Fig. 3. Formation of a bis-sulphonium salt.



X

XI

Fig. 4. Zig-zag conformation of sugar mercaptals.

at the stage corresponding to the methyl ester of the tetramethyl acid, since by submitting tetramethyl gluconolactone to the action of silver oxide and methyl iodide in the presence of water, methyl tetramethyl gluconate was readily obtained in one operation. The fully methylated hexonic ester could be obtained by submitting the ester to five methylations. Haworth(12) carried the reaction a stage further and converted the methyl pentamethyl gluconate to pentamethyl gluconamide. By subjecting this compound to a Weerman degradation, he was able to prepare tetramethyl-aldehydo-D-arabinose.

Haworth's method(12) for the preparation of fully methylated aldehydo sugars involves many steps, whereas the sequence via the mercaptal intermediate is a three step preparation, viz. mercaptalation, methylation and demercaptalation. An obvious choice for the preparation of fully methylated aldehydo sugars would, therefore, be that one involving the mercaptal intermediate. The object of the present work is to attempt to prepare fully methylated aldehydo sugars of D-xylose and L-arabinose via the mercaptals.

OUTLINE

I. Preparation of Mercaptals.

Only diethyl mercaptals were used in this study because, of all the mercaptans, ethyl mercaptan has been one of the most commonly used in the preparation of sugar mercaptals.

L-Arabinose diethyl mercaptal was prepared by Fischer's method(1) which consists of the treatment of L-arabinose with ethyl mercaptan in the presence of concentrated hydrochloric acid. The addition of ice to the reaction mixture gave a yellow crystalline mass which was filtered and recrystallised from ethanol.

In the preparation of D-xylose diethyl mercaptal, the reaction mixture was diluted with tetrahydrofuran and neutralised with barium carbonate. The dark brown sirupy mercaptalation product was distilled to give a pale yellow viscous sirup. Wolfrom, Newlin and Stahly(13) have prepared crystalline D-xylose diethyl mercaptal by acetylating the sirupy mercaptalation mixture and then deacetylating the crystalline acetate. Zinner, Rembarz, Linke and Ulbricht(14) have reported the preparation of crystalline D-xylose diethyl mercaptal by Fischer's method by neutralising the reaction mixture with ion exchange resin.

Attempts to crystallise D-xylose diethyl mercaptal by Wolfrom's method(12) did not proceed with the facility suggested. While attempting to crystallise D-xylose diethyl mercaptal tetraacetate, the pale yellow sirup which was distilled from the mercaptalation reaction product crystallised after being seeded and allowed to stand in the refrigerator

for one week. Further batches of D-xylose diethyl mercaptal were prepared by Fischer's method and the sirupy reaction product, which remained after the removal of tetrahydrofuran, was diluted with isopropyl alcohol and pet. ether added until opalescence just occurred. After allowing the solution to stand in the refrigerator over night, small shiny crystals of D-xylose diethyl mercaptal settled out.

II. Methylation Experiments.

In a recent publication by Kuhn(15), a mixture of strontium oxide and strontium hydroxide was used in place of the reagent silver oxide in methylation experiments. In view of the success of his findings, exploratory experiments were carried out in the hope that this catalyst may be used in our future methylation experiments. Attempts were consequently made to prepare methyl hepta-O-methyl-lactoside from lactose using Kuhn's technique(15).

(a) Preparation of Methyl Hepta-O-methyl-lactoside.

Lactose was methylated using dimethylformamide as solvent in the presence of strontium oxide, strontium hydroxide and methyl iodide. Chromatography of the reaction product on an alumina column resulted in the separation of fully from partially methylated material. Assessments of the degree of methylation were made by observations in the infrared spectrum at λ_{max} 2.90μ. The absence of any absorption band in this region was taken as an indication of the complete etherification of all free hydroxyl groups. Although the melting point of methyl hepta-O-methyl-lactoside

did not correspond to literature values, it was felt that strontium oxide and strontium hydroxide would serve as efficiently as the reagent, silver oxide, in methylation experiments.

(b) Methylation of L-Arabinose Diethyl Mercaptal.

Methylation experiments on L-arabinose diethyl mercaptal were carried out using dimethylformamide as solvent in the presence of a mixture of strontium oxide, strontium hydroxide and methyl iodide. In the first experiment the reaction was carried out at 40°C for a period of 6 hours, while in the second experiment the reaction temperature was changed to 50°C and the reaction time to 18 hours. After thoroughly drying the reaction products on the vacuum pump, the infrared spectra of each sample showed the presence of hydroxyl absorption bands suggesting that the complete methylation of all free hydroxyl groups had not been accomplished. Chromatography on an alumina column failed to give any tetramethyl L-arabinose diethyl mercaptal, for each fraction showed the presence of free hydroxyl group(s) in the infrared absorption spectrum.

Further attempts to prepare tetramethyl L-arabinose diethyl mercaptal were carried out using the Kuhn procedure(16). The infrared absorption spectrum of the purified product showed the presence of free hydroxyl group(s).

(c) Methylation of L-Arabinose Diethyl Mercaptal Under Nitrogen.

Apart from the fact that the sulphides combine with alkyl halides to form crystalline sulphonium salts (e.g. V) comparable to oxonium salts,

and also react with chlorine, bromine or iodine to form dihalides, they are relatively easily oxidised by nitric acid, hydrogen peroxide etc. to the sulphone. Hough(17) reported that diethyl dithioacetal derivatives of monosaccharides can be converted to disulphones on oxidation. The nature of the reaction product depends upon the conditions for oxidation and the type of monosaccharide. These disulphones are useful intermediates in the preparation of the next-lower aldose by the loss of diethylsulphonyl methane(Fischer-MacDonald degradation). The susceptibility of these mercaptals to aerial oxidation is unknown and since these methylations were carried out for periods up to 9 days, it was felt that oxidation of the mercaptal to the sulphone might occur. Since these methylations are carried out under basic conditions, these sulphones could undergo degradation to the next-lower sugar. To exclude the possibility of this side reaction occurring, it was decided to carry out methylations under a nitrogen atmosphere.

L-Arabinose diethyl mercaptal was methylated by the Purdie procedure(18) for three days in an atmosphere of nitrogen. The crude reaction product partially crystallised and the melting point of the crystals agreed with that of the starting materials. The mixture was methylated a further three days by the same procedure. The infrared spectrum of the product showed the presence of an hydroxyl absorption band. Paper chromatographic examination of the acid hydrolyzate of a small sample of the reaction product showed several components with a lower degree of methylation. The crude methylation product was subjected to a third methylation under the same conditions as the two previous methylations. The hydroxyl absorption

band in the infrared was weaker than that after the second methylation. Paper chromatographic examination of the acid hydrolyzate of a small sample of this product still showed the presence of partially methylated material.

(d) Methylation of D-Xylose Diethyl Mercaptal under Nitrogen.

D-Xylose diethyl mercaptal was methylated twice in the presence of methyl iodide, silver oxide and dimethylformamide in a nitrogen atmosphere. The infrared spectrum of each methylation product showed the presence of free hydroxyl group(s), with the intensity of the absorption band being weaker after the second methylation. Paper chromatographic examination of the free sugars after the second methylation showed the presence of at least six components.

(e) Methylation of L-Arabinose Diethyl Mercaptal by the Levene and Meyer Sequence(9).

Levene and Meyer(9) claim to have prepared pentamethyl-aldehydo-D-glucose by methylating glucose diethyl mercaptal twice by the Haworth procedure(10), followed by one Freudenberg methylation(19). Their methylation sequence was repeated on L-arabinose diethyl mercaptal and after the second Haworth methylation the hydroxyl band in the infrared was only slightly weaker than that after the first methylation. This suggested that the second Haworth methylation could have been omitte. Contrary to the reports of Levene and Meyer(9), the addition of sodium brought about an immediate darkening of the reaction mixture and after 30 minutes, the reaction mixture became black suggesting that much decomposition had taken place. However, in the work up of the reaction mixture much of the color could be removed by filtering through an alumina

column using chloroform as the solvent. The resulting mobile reddish-yellow sirup had an infrared spectrum with an extremely weak hydroxyl absorption band. Paper chromatography of the demercaptalated material showed two fast-running components.

(f) Methylation of D-Xylose Diethyl Mercaptal by the Levene Sequence(9).

The methylation sequence of Levene and Meyer(9) was repeated using D-xylose diethyl mercaptal. The D-xylose diethyl mercaptal was subjected to only one Haworth methylation followed by a single Freudenberg methylation. Blackening of the reaction mixture after the addition of sodium was also noted. The infrared spectrum of the final product showed an extremely weak hydroxyl absorption band. Paper chromatographic examination of the demercaptalated material revealed the presence of two fast-running components.

III. Demercaptalation Experiments.

Through variation of the conditions employed for the hydrolysis in the presence of mercuric compounds, of the thioalkyl groups from aldose or ketose dithioacetals, pyranosides, furanosides, 1-thioglycosides or acetals may be obtained. The complete removal of the dithioacetal group can be accomplished by refluxing the mercaptal with aqueous mercuric chloride. The removal of the mercaptal group may equally well be achieved by hydrolysis in the presence of mineral acids. Both of these methods were used and the only preference for the use of the acid hydrolysis method was the elimination of the tedious isolation of the demercaptalated material from the soluble mercury salts.

IV. Characterisation of the Tetra-Methyl-Aldehydo Sugars.

Repeated unsuccessful attempts to prepare the 2,4-dinitrophenylhydrazones and phenylhydrazones derivatives of both sugars were carried out. The evidence to dated for the existence of an aldehydo sugar is (a) aldehyde absorption band in the infrared, (b) instantaneous decolorisation of potassium permanganate solution, and (c) positive Tollens test. Although the blue color of the Fehling's solution disappeared, it was difficult to say whether or not the test was positive. Mercaptals, themselves, will decolorise potassium permanganate. To decide which group was responsible for test (b), a sodium fusion was carried out on a small sample. The result of this test indicated the absence of sulphur in both samples.

EXPERIMENTAL

Unless otherwise stated, all melting points were taken in an open capillary tube using a concentrated sulphuric acid bath.

Paper chromatography was performed by the descending technique using Whatman No.1 paper. Components were located by spraying p-anisidine-trichloroacetic acid reagent and drying in an oven at 100°-110°C. Hydrolysis of small samples for paper chromatographic examination was carried out as described in experiment XVI(a).

Infrared spectra were taken by a Perkin-Elmer Infracord. Intensities of the absorption bands are described using the following abbreviations: s(strong), m(medium), w(weak), vw(very weak) etc.

I. Preparation of Methyl Hepta-O-methyl-lactoside.

Lactose (0.34g) was dissolved in freshly distilled dimethylformamide (25ml) to which was added strontium oxide (1.6g, SrO), strontium hydroxide (0.6g, $Sr(OH)_2$) and methyl iodide (5ml). The mixture was stirred for 4 hours at a constant temperature of 40°C. After the reaction was complete, the solution was centrifuged and the supernatant set aside. The solid material was washed once with dimethylformamide (25ml) and twice with chloroform (25ml). The organic solvents were washed three times with water and dried over anhydrous sodium sulphate. On removal of the organic solvents under reduced pressure, a reddish-yellow sirup (0.32g) remained. Distillation at 200°±20°C/0.05mm yielded a fraction (0.232g). The clear yellow sirup

Approximate weight of sample used = 500mg.

Solvent		Aliquot(ml)	Weight of Material(mg)
pet. ether	100%	150	0
" "	5% benzene	100	0
" "	20% "	100	0
" "	50% "	100	0
benzene	100%	100	0
"	20% chloroform	100	0
"	50% "	50	5
"	50% "	50	132
"	50% "	50	166
"	50% "	50	96
"	50% "	50	42
"	50% "	50	8
chloroform	100%	150	0
		Total weight recovered	449

Fig. 5. Table showing chromatographic separation of reaction product from experiment I.

was fractionally distilled onto a cold finger to yield a fraction (32mg) at 120°C/0.1mm, λ_{max} 2.90μ (OH,m) and a second fraction (144mg) at 160°-220°C/0.05mm, λ_{max} 2.90μ (OH,m).

The experiment was repeated on a one gram scale to give a dark yellow sirup (1.1g). Approximately 500mg of this sirup was chromatographed on an alumina column. For results, see Table I, page 16.

All the fractions collected using benzene:chloroform in the ratio of 1:1 were seeded. The 93mg fraction crystallised rapidly while the rest crystallised at a slower rate. Recrystallisation from light petroleum ether gave a sample with a m.p. 58°C, λ_{max} 2.90μ (OH,vvw). Haworth and Leitch(23) record m.p. 77°-82°C.

II. Preparation of L-Arabinose Diethyl Mercaptal.

L-Arabinose (22.5g, 0.15mole) was dissolved in concentrated hydrochloric acid (42ml). Ethyl mercaptan (23.3g, 28ml, 0.375mole) was added slowly with vigorous shaking. The reaction was exothermic. The mixture was shaken for 30 minutes at room temperature using a mechanical shaker. Approximately 10g of ice was added to the mixture causing the product to crystallise. The crude product was filtered, washed thoroughly with ice cold water and recrystallised from ethanol. Yield: 17.0g, m.p. 127°C, the mother liquors yielded a further 4g, m.p. 124°C. Fischer(1) records m.p. 124°-126°C.

III. Preparation of D-Xylose Diethyl Mercaptal.

D-Xylose (23g) was dissolved in concentrated hydrochloric acid

(42ml) and ethyl mercaptan (28ml) was added slowly with vigorous shaking. The mixture was shaken for a period of 30 minutes at room temperature and then neutralised with barium carbonate. Approximately 200ml of tetrahydrofuran was added to the mixture and the solid material removed by filtration. After washing the solid residue with a further 100ml of tetrahydrofuran, the organic solvent was dried over anhydrous sodium sulphate. Removal of the tetrahydrofuran by distillation gave a dark brown sirup which did not crystallise.

Wolfrom, Newlin and Stahly(13) have prepared crystalline D-xylose diethyl mercaptal by deacetylation of crystalline D-xylose diethyl mercaptal tetraacetate, which was obtained by acetylating the sirupy product of mercaptalation. In order to obtain crystalline D-xylose diethyl mercaptal, Wolfrom's suggestion was carried out on a small sample of the sirup.

IV. Preparation of D-Xylose Diethyl Mercaptal Tetraacetate.

A sample (7g) of the brown sirup obtained under III was dissolved in pyridine (20ml) at room temperature. The solution was cooled in ice and acetic anhydride (40ml) was added and the mixture held at 0°C for 10 minutes and then allowed to stand over night at room temperature. The reaction mixture was then poured into approximately 500ml of ice and water and allowed to stand for 4 days at room temperature. However, no solid separated as suggested.

V. Crystallisation of D-Xylose Diethyl Mercaptal.

The remainder of the sirup from III was distilled at 180°C/0.15mm

to afford a clear yellow viscous sirup which crystallised from isopropyl/ pet. ether. Yield: 10g, m.p. 54°C. Tanaka(6) reports a m.p. 60°C.

When further batches of D-xylose diethyl mercaptal were prepared, it was found that by thinning the mercaptalation product with isopropyl alcohol followed by the addition of pet. ether until opalescence just occurred and cooling overnight in the refrigerator resulted in the formation of small plate-like crystals.

VI. Methylation of L-Arabinose Diethyl Mercaptal.

L-Arabinose diethyl mercaptal (1g) was dissolved in dimethylformamide (25ml), and strontium oxide (4g), strontium hydroxide (1.5g) and methyl iodide (5ml) added to the solution. The mixture was heated for 6 hours on a water bath at 40°C so as to keep the methyl iodide refluxing gently. At the end of the reaction time, the mixture was filtered and the solid material washed thoroughly with chloroform. The organic solvents were combined and washed three times with cold water and then dried over anhydrous sodium sulphate. Removal of the chloroform by distillation gave a yellow sirup. Yield: 0.483g, λ_{max} 2.90μ (OH,ms), chromatographic separation on an alumina column gave three fractions, (a) 56mg eluted with benzene: chloroform (80:20), λ_{max} 2.90μ (OH,w), (b) 168mg eluted with benzene: chloroform (50:50), λ_{max} 2.90μ (OH,mw), (c) 164mg eluted with chloroform, λ_{max} 2.90μ (OH,m).

The experiment was repeated altering the bath temperature from 40°C to 50°C and the refluxing time increased from 6 hours to 18 hours. The work up of the reaction mixture was modified as follows: the dimethyl-

formamide filtrate and chloroform washings were concentrated to a small volume (5ml) and on the addition of chloroform (10ml) the solid precipitate was removed by filtration. The filtrate was concentrated to a small volume and the process repeated until no further solid material precipitated on the addition of chloroform.

Following these modifications, L-arabinose diethyl mercaptal (1g) gave a yellow sirup (0.62g), λ_{max} 2.90μ (OH,mw). Chromatography on an alumina column failed to bring about a separation of the fully from partially methylated material since the infrared spectra of all fractions showed the presence of hydroxyl absorption bands.

VII. Methylation Of L-Arabinose Diethyl Mercaptal by the Kuhn Procedure.

L-Arabinose diethyl mercaptal was dissolved in dimethylformamide (25ml), and silver oxide (1.5g) and methyl iodide (5ml) added. The mixture was shaken for 12 hours. Further additions of methyl iodide and silver oxide were made every 12 hours. After a total reaction time of 36 hours, the solid material was removed by filtration and washed with chloroform. The reaction mixture was worked up using the modification described under VI. Yield: 0.31g, λ_{max} 2.90μ (OH,mw)

VIII. Methylation of L-Arabinose Diethyl Mercaptal under Nitrogen.

L-Arabinose diethyl mercaptal (2g) was dissolved in dimethylformamide (50ml), and silver oxide (3g) and methyl iodide (10ml) added to the solution. A constant atmosphere of nitrogen in the flask and condenser was maintained by attaching a balloon filled with nitrogen to the outlet of the condenser.

The mixture was refluxed for 3 days at a bath temperature of 50°C and further additions of silver oxide and methyl iodide made every 12 hours. At the end of the reaction time, the solid material was removed and washed five times with 25ml aliquots of chloroform. Evaporation of the filtrate and washings gave a brown sirup which partially crystallised. A few crystals were removed and washed with ethyl acetate, m.p. 130°C (L-arabinose diethyl mercaptal, m.p. 127°C).

The reaction product above was methylated for a further 3 days by the same procedure. Yield: 650mg, λ_{max} 2.90μ(OH,m), paper chromatographic examination of the free sugars (in butanone-water azeotrope) showed approximately 6 components. The crude material was methylated a further time under exactly the same conditions described before. Yield: 200mg, λ_{max} 2.90μ(OH,mw), paper chromatographic examination (in butanone-water azeotrope) of the hydrolyzate of a small sample showed 4 components. The chromatograms in both cases were badly streaked and made interpretation difficult.

IX. Methylation of D-Xylose Diethyl Mercaptal under Nitrogen.

D-Xylose diethyl mercaptal (2g) was methylated twice by the procedure described under VIII. In both cases the infrared spectra showed the presence of free hydroxyl groups. Paper chromatographic examination (in butanone-water azeotrope) of the hydrolyzate of a small sample, after the second methylation showed at least 6 components. Again the chromatogram was streaked making interpretations difficult.

X. Methylation of L-Arabinose Diethyl Mercaptal by the Haworth Procedure.

L-Arabinose diethyl mercaptal (5g) was dissolved in water (20ml) contained in a 1 litre three-necked flask fitted with two dropping funnels and a mechanical stirrer. The flask was placed in a water bath maintained at 70°C. To the flask was added dropwise a 30% sodium hydroxide solution (40ml) and dimethyl sulphate (18ml) so that the rate of addition of the former was twice that of the dimethyl sulphate. The addition of reagents was controlled so that after 30 minutes all of the reagents had been added. Vigorous stirring was maintained for a further 15 minutes.

The reaction mixture was poured into ice and water and extracted thoroughly with ether to give a brown oil. Yield: 2.1g. Further extraction in a continuous chloroform extracter for a period of 36 hours yielded a further 1.8g.

The extracts were combined and methylated a further time by this procedure. Total yield: 3.2g, λ_{max} 2.90μ(OH,m), paper chromatographic examination (in butanone-water azeotrope) of the free sugars showed the presence of a variety of polymethylated material.

XI. Methylation of Reaction Product from X by the Freudenberg Procedure.

The sirup (315mg) from experiment X was dissolved in anhydrous ethyl ether (25ml) and powdered sodium (50mg) added to the solution. The mixture was allowed to reflux gently on a water bath for 6 hours. Methyl iodide (10ml) was added and the reaction allowed to proceed with gentle refluxing a further 6 hours. The solid material was removed by filtration and washed thoroughly with anhydrous ethyl ether. The filtrate and washings

were combined, washed with water until the aqueous layer was clear, and then dried over anhydrous sodium sulphate. Evaporation under reduced pressure yielded a yellow-brown sirup. Yield: 200mg, λ_{max} 2.90μ(OH,vw).

XII. Methylation of D-Xylose Diethyl Mercaptal by the Haworth Procedure.

D-Xylose diethyl mercaptal (5g) was methylated by the procedure described above. Yield: 4.2g, λ_{max} 2.90μ(OH,s).

A second methylation by the same procedure gave 2.5g of crude product, λ_{max} 2.90μ(OH,s).

XIII. Methylation of Reaction Product from XII by the Freudenberg Procedure.

The crude reaction product (1.5g) from experiment XII was dissolved in purified dioxan (25ml) and powdered sodium (250mg) added. The temperature of the reaction mixture was held at 70°C for 6 hours on a water bath. The reaction mixture darkened, deepening in color as the reaction time proceeded. The bath temperature was lowered to 50°C and methyl iodide (20ml) added. The mixture was refluxed for a further 6 hours. The solid material was removed by filtration and washed thoroughly with dioxan. The filtrate and washings were concentrated to a small volume (5ml) under reduced pressure and chloroform (10ml) added. The solid material thrown out of solution was removed by filtration. The filtrate was again concentrated to a small volume and the process repeated until no more solid precipitate formed on the addition of chloroform. Yield: 300mg, λ_{max} 2.90μ(OH,vvw). The material could be purified by distillation at 120°C/0.1mm.

XIV. Large Scale Methylation of L-Arabinose Diethyl Mercaptal.

L-Arabinose diethyl mercaptal (20g) was methylated by the Haworth procedure described under X. Yield: 15g, λ_{max} 2.90μ(OH,s). A further methylation by the same procedure gave a crude product, 13.3g, λ_{max} 2.90μ (OH,ms). A further methylation by the Freudenberg procedure using dioxan as solvent, as described under XIII gave a crude product, 5g, λ_{max} 2.90μ (OH,vvw).

XV. Large Scale Methylation of D-Xylose Diethyl Mercaptal.

D-Xylose diethyl mercaptal (20g) was methylated by the Haworth procedure descibed under X. Yield: 15g, λ_{max} 2.90μ (OH,ms). A further methylation by the Freudenberg procedure using dioxan as solvent, as described under XIII gave a crude product, 6g, λ_{max} 2.90μ(OH,vvw).

XVI. Demercaptalations.

(a) Samples (200mg) were hydrolysed by refluxing on a steam bath for 5 hours with a mixture of ethanol (5ml) and 10% sulphuric acid (1ml). The mixture was extracted with ethyl ether and the ether extract washed thoroughly with a saturated sodium bicarbonate solution and dried over anhydrous sodium sulphate.

(b) Samples (200mg) were dissolved in a 2:1 water:ethanol solution (2ml) and an ethanol solution saturated with mercuric chloride (1ml) added. The mixture was refluxed for 1 hour and the mercury mercaptal salts removed by filtration. The filtrate was concentrated under reduced pressure and the

insoluble salts removed by filtration and washed thoroughly with chloroform. The filtrate and washings were concentrated under reduced pressure and the removal of any solid material was repeated as described above. The chloroform extract was dried over anhydrous sodium sulphate.

XVII. Hydrolysis of Tetramethyl L-Arabinose Diethyl Mercaptal.

The crude sirup (5g) from XIV was demercaptalated by method (a) to give a crude, dark brown product. Much of the color was removed by passing through an alumina column using ethyl ether as a solvent. A small sample was purified by distillation at 120°C/0.1mm, λ_{max} 2.90μ (OH,vw), 5.85μ (H-C=O,s). Total yield of undistilled product, 1.5g, paper chromatographic examination (in n-butanol saturated with water at 5°C) showed 2 components, R_f 0.89 (ms), 0.925 (broad,s)

XVIII. Hydrolysis of Tetramethyl D-Xylose Diethyl Mercaptal.

The sirup (6g) remaining from experiment XV was hydrolysed by method (a). Most of the color could be removed by passage through an alumina column using ethyl ether. A small sample was purified by distillation, 120°C/0.1mm, λ_{max} 2.90μ (OH,vw), 5.85μ(H-C=O,s). Total yield of undistilled, 2.2g, paper chromatographic examination (in n-butanol saturated with water at 5°C) showed 2 components, R_f 0.88 (ms), 0.923 (very diffuse).

DISCUSSION

In 1927, Levene and Meyer(9) claimed to have been able to prepare pentamethyl aldehydo-D-glucose by subjecting D-glucose diethyl mercaptal to two Haworth methylations followed by one Freudenberg methylation. The work described in this thesis is an attempt to prepare tetramethyl aldehydo-L-arabinose and tetramethyl aldehydo-D-xylose by methylating the diethyl mercaptal derivatives. From the methylation experiments carried out, it appears that the resistance of certain hydroxyl groups towards Purdie's reagents is much greater than anticipated.

A mixture of strontium oxide and strontium hydroxide was found to be as effective as silver oxide in methylation experiments. The fact that it was possible to prepare methyl hepta-O-methyl-lactoside by a single methylation bears no relationship to the ease of methylation involving the mercaptals. In the case of lactose, the sugars of this disaccharide are in the cyclic form and the hydroxyl groups in this molecule have a different spatial relationship to each other than when the molecule is in the acyclic form.

It is accepted that the most stable conformation of the polyols and sugar mercaptals is one in which the carbon chain assumes a planar zig-zag form. Using this idea and the phenomena on hydrogen bonding, Tanaka(6) was able to explain the relative reactivities of the hydroxyl groups towards Purdie's reagents. The order of decreasing reactivity was found to be as follows; 2-hydroxyl, 3-hydroxyl and primary hydroxyl. In the case of L-arabinose diethyl mercaptal (e.g. X), the ratio of the

2-, and 5-O-methyl ethers was found to be 30:1. On account of the zig-zag conformation rather strong hydrogen bonding between 3-hydroxyl and 5-hydroxyl is possible. This is probably the reason for the absence of the 3-O-methyl-L-arabinose. In the case of D-xylose diethyl mercaptal (e.g. XI), the 2-, 3- and 5-O-methyl ethers were detected in the ratio of 16:10:1. Thus, we can conclude that in our case it is probably the 4-hydroxyl group which is most resistant to methylation.

The resistance of certain hydroxyl groups to alkylation has been recognised for a long time. Irvine and Paterson(20) reported much difficulty in preparing hexamethyl mannitol from 2,3,4,5,6 pentamethyl mannitol using Purdie's procedure. The resistance of a primary hydroxyl group to etherification is contrary to the general trend of reactivities of the hydroxyl groups.

Pryde(11) reported that to convert methyl tetramethyl gluconate (e.g. VIII) to methyl pentamethyl gluconate (e.g. IX) required no fewer than five methylations. The fact that the fully methylated hexonic ester is obtained only after extensive methylation reveals the resistance of some of the hydroxyl groups. The scheme outlined on page 28 is part of the investigations of Haworth(12) to prepare methylated sugars. He studied the effect of sodium hypochlorite on seven methylated sugar acid amides and found that with a methylated sugar amide as with an unmethylated amide the ultimate product was a sugar lower in series. As one of his examples Haworth showed that when 2,3,4,5,6 pentamethyl gluconamide (e.g. VI) was treated with Weerman's reagent, the resulting product was 2,3,4,5 tetra-methyl-D-arabinose (e.g. VII). This method has its limitations for the

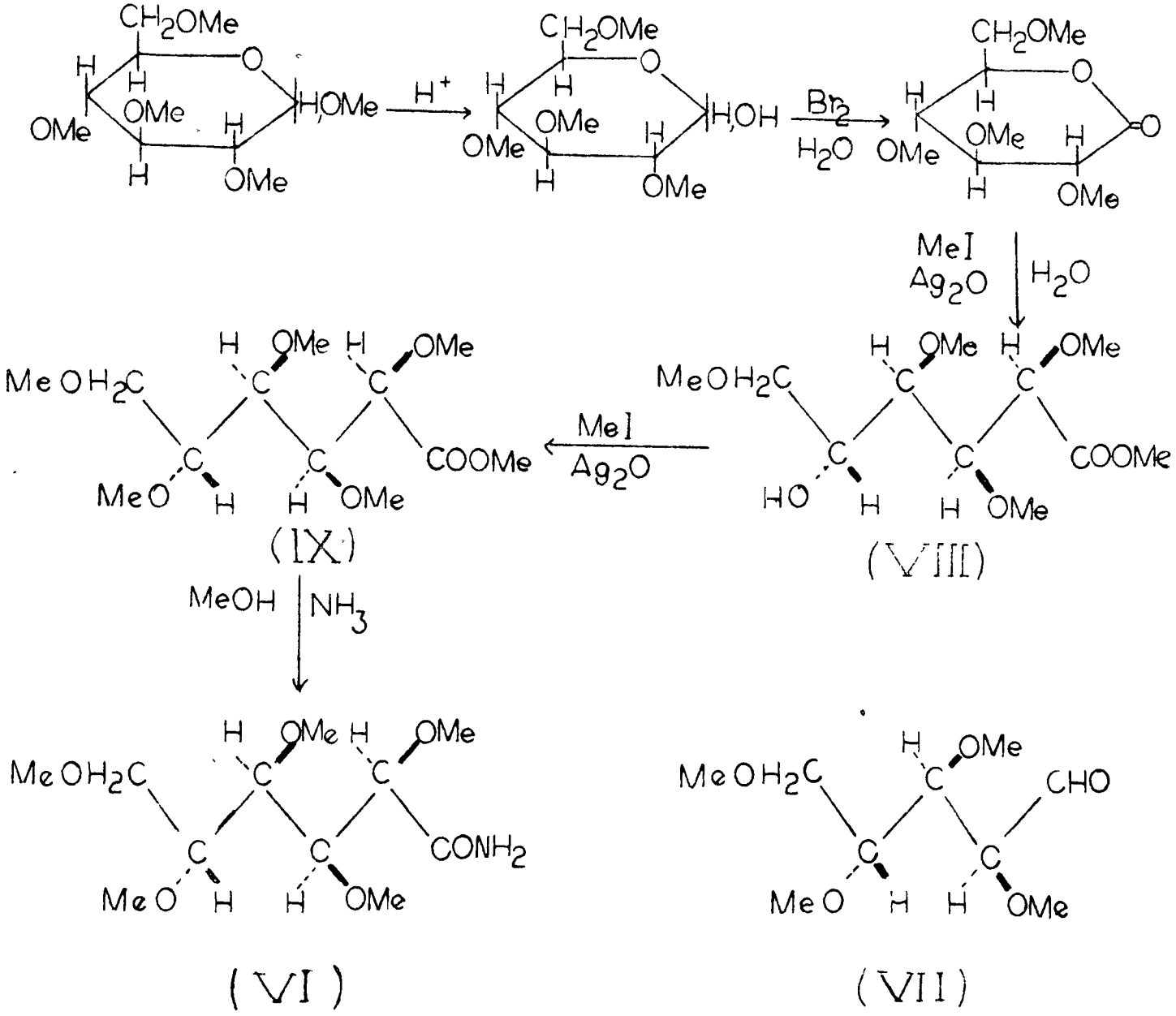


Fig. 6. Preparation of 2,3,4,5 tetramethyl-D-arabinose.

synthesis of a pentamethyl-aldehydo-hexose, since it would require the synthesis of a hexamethyl septanose amide. The septanoses are not readily available materials. Apart from this fact, the reaction sequence involves many steps and it appears that a better method for the synthesis of fully methylated aldehydo sugars would be via the mercaptal intermediate.

Without a knowledge of the mechanism of the Purdie methylation, it would be difficult to pinpoint the exact reason for the resistance of certain hydroxyl groups. However, there are certain factors which are undoubtedly contributing to the resistance of these hydroxyl groups and they cannot be overlooked. Tanaka(6) has pointed out the possibility of hydrogen bonding and its effect on reactivity. Steric hindrance becomes more prominent in view of the work of Bragg and Hough(21). They estimated the uptake of hydrogen from borohydride in a borate buffer by different monosaccharides and have been able to show that 3-O-substituted aldoses and 4-O-substituted hexuloses react more slowly because of steric hindrance (fig. 7). Thus, if a substituent on carbon-3 can exert a deactivating effect on carbon-1, then it follows that a substituent on carbon-1 can sterically reduce the reactivity of the 3-hydroxyl. The resistance of the hydroxyl group in formula VIII can probably be attributed to steric hindrance.

A possible side reaction which warrants consideration when using dimethylformamide as solvent, is the interaction of this solvent with the alkyl halide. Neumeyer and Cannon(22) reported the isolation of tetramethyl-ammonium bromide and dimethylammonium bromide by treating dimethylformamide with methyl bromide in a sealed tube for 6 days at 80°C. Although it is

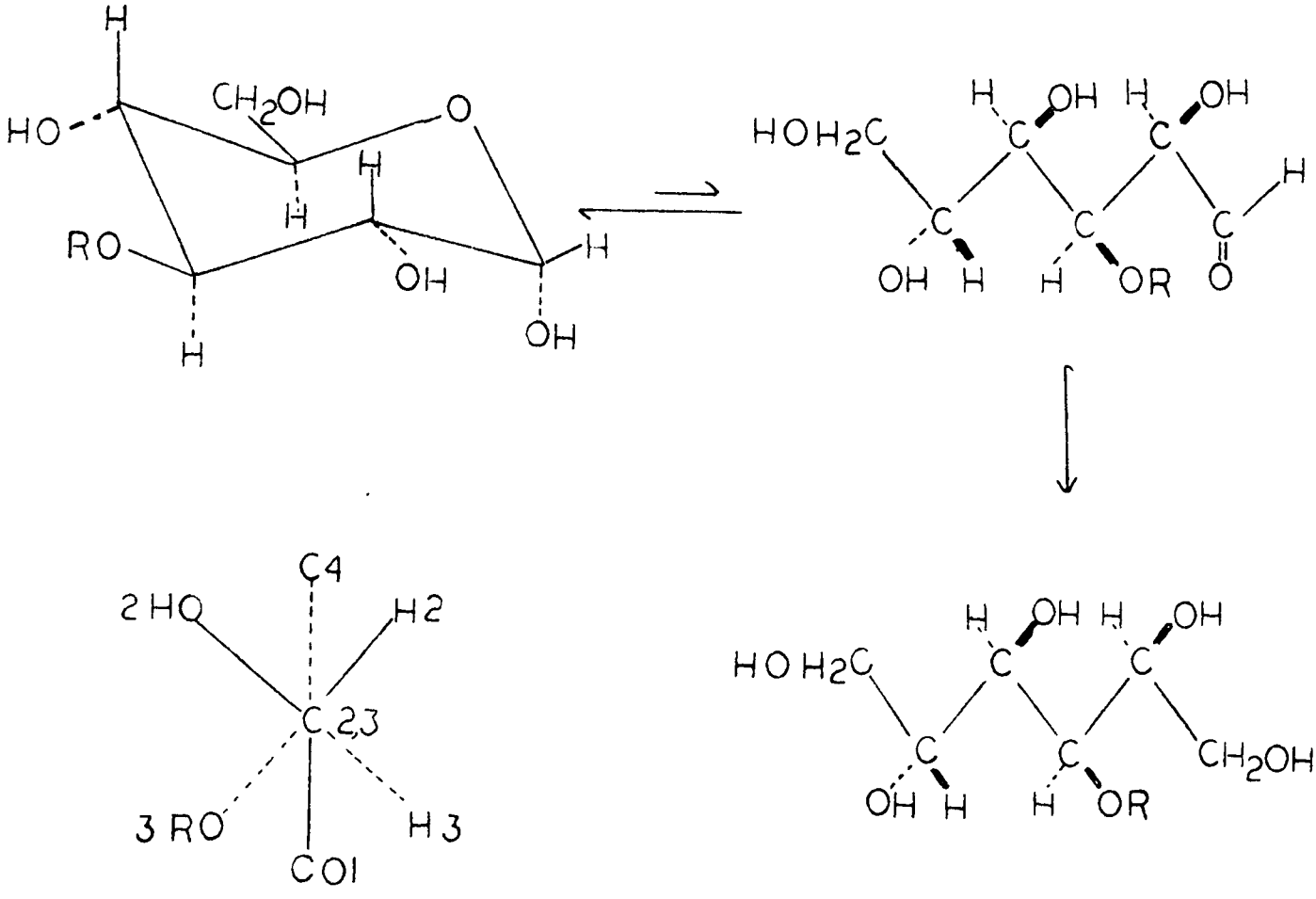


Fig. 1. Conformation of 3-O-substituted aldoses showing possible steric hindrance to hydrogen uptake.

not known whether the corresponding ammonium iodide compound is formed, it is a possibility which should be seriously considered.

When L-arabinose diethyl mercaptal and D-xylose diethyl mercaptal were subjected to two Haworth methylations followed by one Freudenberg procedure, a higher degree of methylation was obtained than when those compounds were methylated in the presence of methyl iodide and dimethylformamide. However, when we consider the exceptionally high resistance of certain hydroxyl groups, especially those of the acyclic sugar derivatives (11, 20), it would be better to use another methylation procedure.

BIBLIOGRAPHY

(1) Fischer, E., Ber., 27: 673 (1894).

(2) Lieser, T., and Leckzyck, E., Ann., 511: 137 (1934).

(3) Dutton, G.G.S., and Yates, K., Can. J. Chem., 550: 36 (1958).

(4) Wolfrom, M.L., Lemieux, R.U., Olin., S.M., and Weisbalt, J. Amer. Soc., 4057: 71 (1949).

(5) Barker, S.A., Bourne, E.J., and Whiffen, D.H., J. Chem. Soc., 3865 (1952).

(6) Tanaka, Y.T., Ph.D. Thesis, University of British Columbia, October (1961).

(7) Foster, A.B., Ann. Rev. Biochem., 45: 30 (1961).

(8) Papadakis, P.E., J. Amer. Chem. Soc., 3465: 52 (1930).

(9) Levene, P.A., and Meyer, G.M., J. Biol. Chem., 175 (1926).

(10) Haworth, W.H., J. Chem. Soc., 107: 8 (1915).

(11) Pryde, J., J. Chem. Soc., 520: 125 (1924).

(12) Haworth, W.N., et.al., J. Chem. Soc., 113: 1975 (1938).

(13) Wolfrom, M.L., Newlin, M.R., and Stahly, E.E., J. Amer. Chem. Soc., 4379: 53 (1931).

(14) Zinner, H., Rembarz, G., Linke, H.W., and Ulbricht, G., Ber., 1761: 90 (1957).

(15) Kuhn, R., et.al., Angew. Chem., 805: 72 (1960).

(16) Kuhn, R., Trischman, H., and Bow, I., Ang. Chemie., 67: 32 (1955).

(17) Hough, L., J. Chem. Soc., 970 (1956).

(18) Purdie, T., and Irvine? I.C., J. Chem. Soc., 83: 104 (1903).

(19) Freudenberg, K., and Hixon, R.M., Ber. dent. chem. Ges., 56: 2125 (1923).

(20) Irvine, J.C., and Patterson, B.M., J. Chem. Soc., 915: 105 (1914).

(21) Bragg, P.D., and Hough, L., J. Chem. Soc., 4347 (1957).

(22) Neumeyer, J.L., and Cannon, J.G., J. Org. Chem., 4681: 26 (1961).

(23) Haworth, W.N., and Leitch, G.C., J. Chem. Soc., 113: 188 (1918).